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## THE PRODUCTION OF BENEFICIAL MUTATIONS IN BARLEY BY IRRADIATION<sup>1</sup>

L. H. SHEBESKI<sup>2</sup> AND T. LAWRENCE<sup>3</sup>

*University of Saskatchewan, Saskatoon, Sask.*

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### ABSTRACT

In view of a report from Svalof that stiff straw in barley could be produced at will by radiation, an induced mutation program was initiated by treating the variety Montcalm with various sources of radiation.  $P^{32}$  was used in 14 dosage-treatment combinations with dosages ranging from 16.25 millirutherfords initial per seed to 2396 millirutherfords initial per plant. A betatron operating at 23 MeV was used for the treatment of seeds at dosages ranging from 5092 to 7130 r equivalents of high-energy X-rays. Radium-beryllium was used for the treatment of both wet and dry seeds with gamma rays and also with neutron + gamma rays in amounts ranging from 5,000 to 15,000 r equivalents.

The greatest percentage of chlorophyll aberrants was observed in the betatron treated Montcalm. The percentage of chlorophyll aberrants appearing in the gamma and neutron + gamma treatments was about the same as the percentage reported for X-ray treated barley. The most favourable proportion of vital to chlorophyll mutations was observed in the  $P^{32}$  treated Montcalm.

A number of stiff-strawed mutants were produced and appeared promising in preliminary tests. Other mutations of interest included several with early maturity and one with dense spikes. The usefulness of the vital mutations obtained needs further evaluation.

### INTRODUCTION

Plant breeders in North America have paid little attention to the development of new varieties in cereal crops by means of induced mutations. The reason may be that steady improvement of cereals has been obtained by systematically and scientifically exploiting the seemingly inexhaustible variability of germplasm available in nature.

Although plant breeders have been successful in developing high yielding varieties of barley with excellent malting quality, they have been less successful in developing varieties that combine yield and quality with strength of straw and non-shattering. Therefore a method by which increased strength of straw in barley can be produced at will, as reported by Guðafsson and MacKey (8), seemed to merit further investigation. They reported that if barley is irradiated with 10,000 r units of X-rays about 500 chlorophyll aberrants and 20 erectoides (stiff-strawed) mutants will appear in the offspring of 10,000  $X_1$  spikes. About one-fourth of the erectoides mutants will equal or excel the parent in yield.

<sup>1</sup> Contribution from the Department of Field Husbandry, University of Saskatchewan.

<sup>2</sup> Associate Professor of Field Husbandry.

<sup>3</sup> Graduate Assistant in Cereal Breeding.

Many reports have appeared in the literature dealing with various aspects of X-radiation. The literature has been reviewed by Gustafsson (6), Gustafsson and MacKey (8) and L. Smith (12).

The mutative values of neutrons and X-rays have been compared by a number of workers. Giles (5) and Thoday (15) presented evidence that neutrons are more efficient than X-rays in producing chromosome aberrations. MacKey (10) reported that there was no difference between X-rays and neutrons for the production of various chlorophyll deficiencies but that upon treatment of growing plant and animal tissue, survival was lower after neutron treatment than after equivalent X-ray treatment. With dormant seeds, however, the results were reversed, i.e., seeds had a higher survival after neutron irradiation.

The use of radioactive phosphorous,  $P^{32}$ , as a source of radiation for the production of mutations has been reported by Spinks *et al.* (13), Arnason *et al.* (1, 2, 3), Ehrenberg *et al.* (4), Thompson *et al.* (16) and Gustafsson *et al.* (7).

Regehr *et al.* (11) reported on the induction of mutation by high-energy X-rays produced by a 23 MeV betatron. They stated that the high-energy radiations were only 0.59 as efficient as ordinary X-rays for the production of lethal mutations in *Drosophila*.

The role of induced mutations in a plant breeding program has been given little attention except by the Swedish plant breeders. Stadler (14) and more recently Hagedoorn (9) appeared doubtful about the practical value of radiations in plant breeding. Gustafsson (6) and Gustafsson and MacKey (8) have described the different mutant types obtained in irradiation experiments with barley, wheat, oats, flax and other agricultural plants. A number of useful mutations were obtained.

Since one of the objectives in the barley breeding program at Saskatoon is the production of a high-yielding malting variety suitable for straight combining and since excellent facilities are available for inducing mutations, an induced mutation program was initiated in 1950 to try to improve the straw strength of Montcalm (the leading malting variety of Western Canada). This paper is a report of the methods of approach and the results thus far obtained.

#### MATERIALS AND METHODS

Montcalm, the variety used in this study, is a blue-seeded, six-rowed, smooth-awned barley with a moderately weak straw. It is susceptible to stem and leaf rust but moderately resistant to covered smut. During the breeding of this variety by its originator, E. A. Lods, at Macdonald College, Que., it was selected to a high state of purity and may be considered quite uniform.

Various types of radiation were used to induce mutations in Montcalm.  $P^{32}$  was used in 14 dosage-treatment combinations. Radium-beryllium was used as a source for six dosage-treatment combinations of neutron + gamma rays and also for six dosage-treatment combinations of gamma rays by excluding the neutrons with a cadmium screen. A betatron operating at 23 MeV was used to obtain three dosages of high-energy X-rays.



The  $P^{32}$  treatments were carried out in two ways. The first was to push dry seed into soil to a depth of two inches and then to pour measured quantities of  $P^{32}$  with carrier  $P^{31}$ , in the form of  $H_3PO_4$  solution, on the seed in the soil. Later, at different dates before pollen mother cell formation, the crowns of some of the plants were uncovered and retreated by pouring measured quantities of  $P^{32}$  with carrier  $P^{31}$  in solution over them. The soil was then replaced and the plants were watered. The second method of  $P^{32}$  treatment was to soak seeds in  $P^{32}$  solution (carrier-free) in Petri dishes and then to plant them in moist soil. The pertinent information for the 14 dosage-treatment combinations is summarized in Table 1.

TABLE 1.—THE NUMBER OF SEEDS THAT WERE TREATED WITH  $P^{32}$  AND THE DOSAGE THAT WAS APPLIED IN MILLIRUTHERFORDS (MRD.)\* PER SEED OR PLANT AT THE TIME OF TREATMENT

Treatment designation	Number of seeds	Treatment in mrd. related to date of planting					Time in solution	Total treatment in mrd. initial per seed or plant
		1st day	30th day	34th day	45th day	49th day		
1A	51	111	—	585	—	1700	—	2396
1B	50	111	—	585	—	—	—	696
1C	300	111	—	390	—	—	—	501
1D	33	111	—	—	—	—	—	111
2A	25	280	585	—	—	—	—	865
2B	200	280	390	—	—	—	—	670
2C	500	280	195	—	—	—	—	475
2D	240	280	—	—	—	—	—	280
2E	160	280	—	—	850	—	—	1130
2F	40	280	—	—	1700	—	—	1980
2G	10	—	—	—	1700	—	—	1700
3A	400	—	—	—	—	—	5 days	32.50
3B	200	—	—	—	—	—	5 days	16.25
4	500	—	—	—	—	—	88 hours	29.00

\* One mrd. equals 1000 disintegrations per second.

Plants from several of the treatments were sampled in  $X_1$  (the treated generation), to determine the uptake of  $P^{32}$  from the soil. A number of treated plants were dug up, washed, wet ashed, and the  $P^{32}$  content of the plants calculated for the day of removal of the plants from the field. Allowance was made for the decay of the  $P^{32}$  and then the percentage uptake for the treatments sampled was calculated.

Approximately 12,000 seeds were treated with gamma rays and 12,000 with neutron + gamma rays using radium-beryllium as a source for irradiation. Dosages of 5,000, 10,000 and 15,000 Röntgen equivalents of each type of radiation were given. For each of the six treatments 2,000 dry seeds and 2,000 seeds which had been soaked in water for two hours were irradiated.

The rates of treatment and the number of seeds treated with high-energy X-rays produced by the betatron were as follows: 100 seeds at 5092 r equivalents; 104 seeds at 6290 r equivalents, and 150 seeds at 7130 r equivalents.

All treated material was grown on the Field Husbandry Cereal Investigation Plots at Saskatoon in 1950. The plants from each treatment were pulled and wrapped separately. Each of the plants was threshed on a tiller rather than on a plant basis. Approximately 27,000  $X_1$  plants were harvested and these averaged more than five tillers per plant so that over 135,000 heads were threshed. Each treatment was sampled and a very small proportion of the possible  $X_2$  population was grown in the Field Husbandry greenhouses during the winter of 1950-51.

During the summer of 1951, on approximately 3 3/5 acres of land, 17,046 tiller progenies representing 5,368  $X_1$  plants were grown in rows five feet long. In addition, approximately 1,000  $X_3$  progenies from  $X_2$  plants grown in the greenhouse the previous winter were grown in rows 11½ feet long. Untreated Montcalm was grown in every tenth row throughout the entire nursery as a check.

During the winter of 1951-52 seed of one of the stiff-strawed mutants that was found the previous summer was increased in California (courtesy of the Canada Department of Agriculture). In 1952 the mutant was compared with the parent Montcalm at five locations and malting quality results were obtained for one location.

## EXPERIMENTAL RESULTS

### Uptake of $P^{32}$

The usefulness of  $P^{32}$  for the production of mutations must be dependent in part on the uptake of  $P^{32}$ . Some of the treatments listed in Table 1 were sampled and activity counts were made. A summary of the amounts of  $P^{32}$  in the counted plants is presented in Table 2.

TABLE 2.—THE  $P^{32}$  UPTAKE IN MRD. AND IN PER CENT FOR THE TREATMENTS SAMPLED

Treatment	Number of plants counted	Mrd. $P^{32}$ available per plant at collection date	Average plant uptake in mrd. at collection date	Average plant uptake in per cent of supplied $P^{32}$
1A	5	1408.8	146.4	10.4
1B	10	364.4	5.3	1.5
1D	10	37.5	0.006	0.016
2D	11	115.4	0.039	0.034
2G	5	1193.7	58.0	4.9

The highest recorded uptake was obtained when applications of  $P^{32}$  were repeated, and the largest application was made at approximately the time of pollen mother cell formation.

### Observed Effects of Treatments in $X_1$

During the threshing of the  $X_1$  plants, the heads were carefully examined for observable differences from typical Montcalm. The most



striking off-types observed were twin heads, one of which was found in the  $P^{32}$  3A treatment and three of which were found in the 15,000 r neutron + gamma wet treatment. Seed from these plants gave rise to normal appearing progeny.

A double kernel (two kernels joined under one seed coat) was found in the  $P^{32}$  1C treatment. From this double kernel a stiff-strawed mutant was produced which bred true for stiff straw in later generations.

A number of plants were observed to be four-rowed rather than six-rowed, but their progeny appeared normal.

Except for the few observed differences mentioned, practically no other effects of the radiation treatments were noticed in  $X_1$ .

#### Observed Effect of Treatments in $X_2$ and $X_3$

The "off-types" which were found were classified either as chlorophyll mutants or vital mutants. The vital mutants were observed after the field headed. A brief description of each type of mutant, and the  $X_3$  behaviour of each that was grown in  $X_3$ , follows.

##### *Chlorophyll Mutants*

In  $X_2$  the first obvious effects of radiation were the chlorophyll deficient mutants which appeared in the seedling stage, and most of which were lethal. The lethals included albinos, yellows, stiff yellows, whites with green tips and yellows with green tips. The non-lethals included striped seedlings, pale-green seedlings and a variegated plant which developed albino heads and seed. Invariably, the mutants appeared in the progeny of only one tiller of the  $X_1$  plants.

##### *Early-maturing Mutants*

The mutants for earliness headed and matured a week to two weeks earlier than the checks. They were found in five different plant progenies. An early-maturing, rough-awned plant was found in the  $P^{32}$  2B treatment. A somewhat similar type was found in the  $P^{32}$  3A treatment. Two smooth-awned, early-maturing mutants, one of which was short-strawed, were found in the 15,000 r neutron + gamma wet treatments. The fifth early-maturing mutant was found in the 15,000 r gamma dry treatment.

Three of the mutants were grown in  $X_3$  and each bred true for earliness and other plant characters. Two of the three were rough-awned and they bred true for rough awn as well as for earliness. One of these and the smooth-awned mutant were earlier-maturing under greenhouse conditions than the commonly grown early-maturing varieties, Olli and Warrior.

One of the rough-awned, early-maturing mutants yielded sufficient seed for a malting prediction test. There was no apparent quality difference shown between the mutant and the adjacent Montcalm check.

##### *Two-rowed Mutants*

Three two-rowed plants were found. One was located in the  $P^{32}$  2D treatment, one in the 10,000 r neutron + gamma dry treatment and one in the 10,000 r neutron + gamma wet treatment. Two of the two-rowed plants were rough-awned and their progeny segregated for two- and six-row and for rough and smooth awn in  $X_3$ . The progeny of the two-rowed plant

found in the 10,000 r neutron + gamma wet treatment were all smooth-awned but segregated for the two- and six-row character and also for blue and yellow aleurone.

### *Compactoid Mutant*

Several plants with compact heads were found in one tiller progeny row in the 15,000 r neutron + gamma wet treatment. The dense-headed type bred true in  $X_3$  and appeared to be very stiff-strawed.

### *Stiff-strawed Mutants*

A number of plant progenies from several of the treatments were found to have considerably stronger straw than that of the adjacent Montcalm checks. They appeared to breed true for the stiff-straw characteristic in  $X_2$  and  $X_3$ . The stiff-strawed mutants were easily detectable in low spots because they did not lodge when lodging occurred in the surrounding progeny rows and checks. In the portion of the field where lodging did not occur the mutants could be distinguished from the Montcalm checks by the erectness of their spikes at maturity. One of the stiff-strawed mutants was grown in  $X_3$  in the field and a prediction test for quality was obtained along with a prediction test for an adjacent Montcalm check. No quality difference was shown between the mutant and Montcalm.

### *Hulless Mutant*

One of the plants grown in the greenhouse in  $X_2$  from the betatron 6290 r treatment appeared to be very loose hulled. In  $X_3$  segregation took place for hulless and hulled seeds.

### *Other Mutants*

Other mutants observed included several rough-awned plants, a multi-florous plant, some late-maturing plants, plants with leafy bracts, and plants with head deformities such as "tweaky top" and twin heads. Sterile and semi-sterile plants were observed in large numbers scattered throughout the field. A number of dwarf plants were also found. No counts were made on the numbers of dwarfs and steriles.

### **Distribution of Mutations as to Treatment.**

A summary of the distribution per treatment of the plant progenies which showed chlorophyll aberrants or vital mutations is presented in Table 3. Only the treatments in which mutations were recorded are included in the table.



TABLE 3.—THE DISTRIBUTION OF MUTATIONS IN RELATION TO TREATMENT AND SOURCE OF RADIATION

Source	Treatment	Number of plant progenies grown	Number of progenies with chlorophyll deficiencies	Number of progenies with vital mutants	Per cent chlorophyll deficiencies	Per cent vitals
$P^{32}$	1C	292	2	1	0.68	0.34
	2B	192	2	1	1.04	0.52
	2C	462	2	0	0.43	0.00
	2D	237	1	2	0.42	0.84
	2E	153	1	0	0.65	0.00
	2F	40	1	0	2.50	0.00
	3A	72	2	1	2.78	1.39
Betatron	5092 r	65	8	0	12.31	0.00
X-rays	6290 r	48	11	1	22.92	2.08
	7130 r	91	12	1	13.18	1.09
Radium-beryllium neutron + gamma	15,000 r wet	594	31	5	5.21	0.84
	10,000 r wet	135	16	2	11.85	1.48
	5,000 r wet	250	1	4	0.40	1.60
	15,000 r dry	632	19	0	3.01	0.00
	10,000 r dry	304	13	2	4.27	0.66
	5,000 r dry	251	14	1	5.57	0.40
Radium-beryllium gamma	15,000 r wet	158	13	0	8.22	0.00
	10,000 r wet	218	11	0	5.04	0.00
	5,000 r wet	238	9	2	3.78	0.84
	15,000 r dry	275	21	4	7.63	1.45
	10,000 r dry	284	9	1	3.16	0.35
	5,000 r dry	98	3	0	3.06	0.00
Totals— $P^{32}$	—	1148	11	5	0.76	0.35
Betatron	—	204	31	2	15.20	0.98
Radium-beryllium	—	3437	160	21	4.65	0.61

The 4.65 per cent of chlorophyll deficiencies from the neutron + gamma and gamma ray treatments was in agreement with the results obtained by Gustafsson and MacKey (8) with X-rays. However, the chlorophyll aberrants resulting from betatron treatments were much more numerous than expected. This indicates that the high-energy rays from the betatron were more potent for this type of mutation than the lower energy X-rays. The results are not in agreement with the findings of Regehr *et al.* (11) in *Drosophila*.

Larger uptakes of  $P^{32}$  than that obtained would be necessary before the value of  $P^{32}$  as a mutagenic agent could be completely assessed. That more work should be done with  $P^{32}$  is indicated by the fact that the most favourable proportion of vital mutants to chlorophyll aberrants was obtained with this source of radiation. Whether or not the favourable proportion of vitals would be maintained with increased uptake of  $P^{32}$  is worth determining.

## AGRONOMIC AND QUALITY RESULTS

Although 20 mutants were given preliminary yield trials in 1952, only one of the stiff-strawed mutants (Sask. 5203) was tested sufficiently to warrant a report. Sask. 5203 was compared agronomically with Montcalm at five locations. Malting quality results were obtained from the test grown at Tisdale. Agronomic and quality data are presented in Table 4.

TABLE 4.—THE AGRONOMIC AND QUALITY DATA FOR THE STIFF-STRAWED MUTANT SASK. 5203 IN COMPARISON WITH THE PARENT MONTCALM

Variety	Sask. No.	Yield in bushels per acre					Average 5 stations
		Edmonton	Saskatoon	Tisdale	Melfort	Indian Head	
Montcalm	2122	55.1	56.6	69.5	31.9	41.6	50.9
Montcalm stiff	5203	62.2	53.5	63.1	30.5	43.1	50.5
<i>Resistance to lodging (scale 1-10, with 1 = strong)</i>							
Montcalm	2122	3.0	3.0	2.5	—	—	
Montcalm stiff	5203	1.0	2.0	1.0	—	—	
<i>Quality results from the Tisdale test</i>							
		1000 k. wgt., gm.	Barley nitrogen, per cent	Wort nitrogen, per cent	Malt extract, per cent	Sacch. activity, ° L.	
Montcalm	2122	40.5	2.52	1.30	74.0	170	
Montcalm stiff	5203	42.0	2.65	1.41	73.4	185	

There were no significant differences in yield at any location between Montcalm and the stiff-strawed mutant. At the three locations where there was a differential in lodging, the stiff-strawed mutant was unquestionably superior to Montcalm in strength of straw. More quality tests will have to be obtained to determine whether there is a difference between the two varieties.

## DISCUSSION

Taking into consideration the time and costs involved, the final evaluation of any program in plant improvement must be dependent on the results achieved. The purpose of the mutation work herein reported was to improve the straw strength of Montcalm without decreasing yield or lowering quality. Whether the purpose has been fully achieved has not yet been determined. Stiff-strawed mutants have been obtained and prediction and malting tests indicate that quality has not been impaired. Comparative yield tests were commenced in 1952 and the results of several years of such trials should show whether the project has been fully or only partially successful.



In addition to the strong-strawed mutants the early-maturing mutants may be of considerable practical value. Early-maturing varieties of good malting quality are desirable for areas with a short growing season and also for areas where delayed seeding is practised for weed control.

In 1952, two years after the initiation of the mutation program, 20 mutants were entered into yield trials. On the basis of the results achieved in this short period, the authors believe that radiation may have a place in a well-rounded plant breeding program. However, it is felt that further work is necessary on methods of inducing mutations.

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# A STUDY OF THE EFFECT OF THE FINENESS OF GRINDING GRAINS ON THE EFFICIENCY OF ALL-MASH RATIONS FOR LAYING HENS<sup>1</sup>

T. M. MACINTYRE<sup>2</sup> AND M. H. JENKINS<sup>3</sup>

*Dominion Experimental Farm, Nappan, N.S.*

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## ABSTRACT

All-mash rations of three different degrees of granulation were fed to laying hens in pens and laying batteries. The degree of fineness of the rations was determined by use of standard screens. The birds fed the more finely ground rations showed improved feed efficiency. Individual egg production was not significantly affected by the granulation of the ration. On being presented with a choice between coarse, medium and finely ground rations, birds showed a preference for coarsely ground feed.

The all-mash system of feeding laying hens has been the subject of considerable study among poultrymen (4, 7, 8, 9, 10). Much of this work has taken the form of a direct comparison of all-mash rations vs. grain and mash rations, with little attention being paid to the size of feed particles present in the all-mash ration. The work of several investigators indicates that birds show a preference for coarsely ground feed (6, 1). However, reports of the effect of feed particle size on egg production and weight gains are conflicting, some workers reporting improved performance from coarsely ground feeds (1), and others reporting no differences between coarsely and finely ground feeds (5, 2).

Much of the work reported in studies of this nature is difficult to interpret and it is almost impossible to make practical application of the findings because standard measures of the degree of fineness of the rations are not given. Frequently grain and mash rations were fed rather than all-mash rations and this would tend to obscure any possible differences due to variations in granulation of the mash.

The experiments reported herein were designed to study the effect of varying the degree of granulation of the grains in an all-mash ration, on the performance of laying hens.

## EXPERIMENTAL

Two experiments were carried out: Experiment 1, with the birds housed in pens, and Experiment 2, with the birds housed in individual laying batteries. The feed was supplied as an all-mash ration in both trials. The compositions of the feed mixtures are presented in Table 1. In each trial the ration was supplied in three different degrees of texture conveniently designated as coarse, medium, and fine. The variation in mash texture was achieved by grinding the grain constituents going into the mixture to the desired degree of fineness before mixing.

Measures of the degree of fineness of the rations are given in Table 2. Taking the total percentage retained on the 20-mesh screen and the ratio of the coarse, medium, and fine particles as a measure of the mash texture,

<sup>1</sup> Contribution from Poultry Division, Experimental Farms Service, Canada Department of Agriculture.

<sup>2</sup> Research Officer.

<sup>3</sup> Technical Officer.



TABLE 1.—FORMULA OF RATIONS

	Experiment No.	
	1 <sup>1</sup>	2 <sup>2</sup>
	%	%
Oats	29.0	35.0
Wheat	35.0	30.0
Barley	22.5	15.0
Corn (Yellow)	—	5.0
Wheat bran	2.5	—
Wheat shorts	—	5.0
Lay mix <sup>3</sup>	—	10.0
White-fish meal	5.0	—
Vita-grass	2.5	—
Bone meal	1.5	—
Oyster shell	1.0	—
Salt	0.5	—
Fish oil (1000A-200D)	0.5	—
	100.0	100.0

<sup>1</sup> This ration contained one-quarter pound of manganese sulphate and one-half gram of synthetic riboflavin per ton.

<sup>2</sup> Sixty pounds of finely ground limestone was added per ton as a source of calcium.

<sup>3</sup> The lay mix was a commercial concentrate containing the necessary protein concentrates, vitamins and minerals.

TABLE 2.—TEXTURE OF RATIONS AS MEASURED BY STANDARD SCREENS

	Screens, meshes per inch	Per cent of total retained by each screen		
		Coarse	Medium	Fine
<i>Experiment 1:</i>	12	48	17	7
	20	30	39	27
	42	10	25	53
	80	6	11	11
	80*	6	8	2
	—	100	100	100
Ratio of coarse, medium and fine particles*	—	5 : 3 : 2	2 : 4 : 4	1 : 3 : 6
<i>Experiment 2:</i>	10	39	28	2
	20	21	23	29
	40	21	26	38
	80	16	20	26
	80*	3	3	5
	—	100	100	100
Ratio of coarse, medium and fine particles	—	4 : 2 : 4	3 : 2 : 5	0 : 3 : 7

\* Coarse particles: Retained on 10- or 12-mesh screen.

Medium particles: Retained on 20-mesh screen.

Fine particles: Pass through 20-mesh screen.

(Based on method of Silver (11)).

the medium and finely ground feeds were of about the same texture in both experiments, but the coarse feed in Experiment 1 was of a coarser texture than that fed as coarse feed in Experiment 2. The difference in texture between the coarse and the medium was greater in Experiment 1 than in Experiment 2.

TABLE 3.—POUNDS OF FEED CONSUMED AND NUMBER OF EGGS PRODUCED PER 100 BIRDS PER DAY BY 28-DAY PERIODS

	Texture of ration	Feeding period										Average
		1	2	3	4	5	6	7	8	9	10	
<i>Experiment 1:</i> Feed consumption	Coarse	24	28	32	34	36	31	29	29	28	—	30
	Medium	24	27	31	34	34	28	28	27	25	—	29
	Fine	23	25	29	31	34	28	29	29	27	—	28
Egg production	Coarse	33	35	53	73	78	71	62	58	55	—	57
	Medium	33	34	49	73	74	69	62	58	55	—	56
	Fine	31	33	50	75	78	71	70	67	62	—	59
<i>Experiment 2:</i> Feed consumption	Coarse	31	28	26	27	28	29	32	33	34	32	30
	Medium	29	28	27	29	30	31	34	35	34	32	31
	Fine	28	27	26	26	27	27	31	32	31	32	29
Egg production	Coarse	26	25	29	34	33	56	66	67	62	60	46
	Medium	26	29	30	38	46	60	63	68	62	56	48
	Fine	20	21	27	30	36	57	63	64	63	58	44



## EXPERIMENT 1

*Procedure*

Experiment 1 was carried out in pens. Each ration of different texture was supplied to 10 pens each of 20 Barred Plymouth Rock hens, a total of 30 pens. The hens were randomized into the laying pens directly from the range. They were all of the same age, from the same parent stock, and raised as one flock. The experiment was carried on for a period of 252 days, beginning in December, 1948.

The all-mash ration was fed ad libitum in hoppers. Oyster shell, grit, and water were kept before the birds at all times.

A record was maintained of egg production, egg weight, feed consumption, and body weight. Egg production and egg weight were recorded daily on a pen basis for all pens, and on an individual basis for four pens on each ration of different texture, for the first 112 days of the trial. Gross feed consumption was determined for each pen by weighting back the unconsumed portion of the feed at 28-day intervals. Average individual feed consumption was determined for each pen by recording mortality and calculating feed consumption on the basis of the actual number of bird days for every 28-day feeding period. The birds were weighed individually at the beginning and end of the experiment.

*Results and Discussion*

Under the conditions of this experiment there were no differences in egg production, average individual egg weight, increase in body weight, or mortality between the birds fed the coarse, medium, and fine rations, but feed consumption was lower on the finer rations, and this was reflected in better feed efficiency (Table 4).

It is not possible to tell from these data whether the differences in feed consumption were due to the fine rations being less palatable than the coarse ration. A palatability test run in conjunction with Experiment 2 showed that birds preferred the coarse ration when they were presented with a choice between the three texture grades.

However, when presented with only one ration, the birds consumed the mash quite readily regardless of texture, although the birds on the fine ration consumed less feed during the first four periods, than did the birds on the medium and coarse rations (Table 3). They also came into lay more slowly but continued at a higher rate of lay after the fourth period. The results of this and other work reported by Eley and Hoffman (5) and Berg and Bearse (2) indicate that, while birds prefer coarsely ground particles, they will readily consume and efficiently utilize coarse or fine rations. In this experiment, indications are that the birds consumed less of the finer rations for some reason other than palatability. The birds on the fine ration were significantly lighter in body weight than the birds on the coarse and medium rations, both at the beginning and end of the experiment and these differences were no doubt partly responsible for the significant differences in feed consumption and feed efficiency between the birds on the fine, and the medium and coarse rations. The significant difference in feed consumption between the birds on the coarse and medium rations must be attributed to more efficient utilization of the medium

TABLE 4.—RESULTS OF FEEDING RATIONS WITH VARYING SIZE GRAIN PARTICLES TO LAYING HENS IN PENS<sup>1, 2</sup>

Criteria of comparison	Granulation of mash			Diff. req. for sig. P = 0.05
	Coarse	Medium	Fine	
Number of birds per pen at start of experiment	20.0	20.0	20.0	
Av. number of birds per pen at end of experiment	17.8	17.8	18.3	
Per cent mortality	11.0	11.0	8.5	
Egg production—				
Av. on a hen house basis	136.0 ± 11.0 <sup>4</sup>	134.0 ± 9.0	142.0 ± 11.0	—
Av. on a survival basis	153.0 ± 12.0	150.0 ± 10.0	156.0 ± 12.0	—
Av. pounds of eggs per bird	19.2 ± 1.4	18.8 ± 0.9	19.5 ± 1.1	—
Feed consumption (pounds) <sup>3</sup> —				
Av. feed cons. per bird	76.5 ± 2.9	72.3 ± 2.4	71.0 ± 2.2	2.4
Av. feed cons. 100 birds per day	30.2 ± 1.2	28.7 ± 1.0	28.2 ± 0.9	0.95
Av. feed cons. per bird (adjusted) <sup>6</sup>	75.5	71.8	72.2	2.0
Feed efficiency for egg production—				
Number of eggs per 100 lb. feed	189.0 ± 9.0	196.0 ± 9.0	210.0 ± 12.0	9.2
Pounds of eggs per 100 lb. feed	25.2 ± 1.3	26.0 ± 1.2	27.4 ± 1.5	1.2
Body weight of birds (pounds)—				
Av. initial body weight	5.49 ± 0.25	5.56 ± 0.22	5.10 ± 0.41	0.27
Av. final body weight	6.97 ± 0.30	6.96 ± 0.32	6.41 ± 0.47	0.31
Av. individual egg weight (gm.) <sup>5</sup>	61.8 ± 4.0	62.0 ± 3.6	60.6 ± 3.5	—
Av. individual egg prod. for 112 days <sup>5</sup>	49.0 ± 24.0	49.0 ± 21.0	51.0 ± 23.0	—

<sup>1</sup> All data with two exceptions (see note 5) are the means of 10 pen averages.<sup>2</sup> Duration of experiment, 252 days.<sup>3</sup> Calculated by correcting for birds that died.<sup>4</sup> Standard deviation.<sup>5</sup> Data from 80 birds on each ration, trapnested for the first 112 days of the trial.<sup>6</sup> Feed consumption adjusted for differences in body weight and egg production.

ration since body weight and egg production were similar. The fact that the birds on the finely ground ration were lighter in body weight than the birds on the other two rations, both at the beginning and end of the experiment, throws some doubt on the validity of any conclusions drawn from the significant differences in feed consumption and feed efficiency between the birds fed the fine and the coarse ration (Table 4). The improved feed efficiency for egg production shown by the birds on the fine ration might be due entirely to the fact that these birds required less feed for maintenance and thus had more feed available for egg production. However, when the feed required for maintenance was calculated according to the method outlined by Brody (3) it was found to be 61.5, 61.2, and 56.9 pounds per bird for the coarse, medium and fine rations respectively, leaving 15.0, 11.1, and 14.1 pounds of feed available for egg production in each case. The amount of feed required above maintenance to produce a dozen eggs was 1.2, 0.9, and 1.0 pounds respectively for the birds on the coarse, medium and finely ground rations, indicating improved feed efficiency for egg production by the birds on the finer rations. The reason for this



increased efficiency is not apparent from these data, but may be due to improved digestibility of the finer feed.

Variation in individual egg production as measured by the standard deviation of the individual egg production of eighty birds on each ration for the first 112 days of the trial was least for the birds on the finer rations.

## EXPERIMENT 2

### *Procedure*

Experiment 2 was carried out in individual laying batteries with Barred Plymouth Rock hens. The birds were randomized into the laying batteries directly from the range. Ninety birds were started on each ration of different texture. The experiment was conducted for a period of 280 days beginning in November 1951. Feed and water were supplied ad libitum.

Individual bird records were maintained for feed consumption, egg production, egg weight, specific gravity of the eggs, and body weight.

The composition of the ration is shown in Table 1. The texture of the rations (Table 2) corresponded quite closely to the texture of the rations used in Experiment 1, except for the coarse mash, which contained a larger percentage of fine particles in this case. Sixty pounds of finely ground limestone was added to each ton of mash to supply adequate calcium for egg production. Insoluble grit was kept before the birds at all times.

The palatability of the three rations used in this experiment was tested by offering a pen of birds a free choice between the three rations over a period of 100 days. Two feed hoppers of each ration were placed in the pen. The hoppers were shifted around at weekly intervals to prevent habit patterns from playing any part in the choice of feed.

### *Results and Discussion*

The hens housed in laying batteries showed no differences in egg production, egg weight, specific gravity of eggs, body weight, or mortality between the various rations fed, but feed consumption was lower on the fine ration (Table 5). When feed consumption was adjusted by regression for varying body weight and varying egg production there was a significant difference between the adjusted means. Thus the birds on the fine ration required less feed to produce the same number of eggs and maintain the same body weight as the birds fed the medium and coarse rations, indicating improved feed efficiency for the birds on the fine ration. However, there was no significant difference in feed efficiency as measured by number and pounds of eggs produced by 100 pounds of feed, on the basis of actual egg production and feed consumption.

Although significant differences are lacking in all criteria measured except feed consumption, trends similar to those of Experiment 1 are evident. The birds fed the fine ration consumed less feed at the start of the experiment and came into production more slowly (Table 3). Variation in individual egg production was least for the birds on the more finely ground ration (Table 5).

When the palatability of these rations was tested, the relative amounts of coarse, medium, and fine feed consumed were in the ratio of 3 : 2 : 1, showing a considerable preference on the part of the birds for the coarse feed. This preference for coarse particles, when a choice of coarse and fine particles are available, is generally accepted as an established fact.

TABLE 5.—RESULTS OF FEEDING RATIONS WITH VARYING SIZE GRAIN PARTICLES TO LAYING HENS IN INDIVIDUAL LAYING BATTERIES<sup>1</sup>

Criteria of comparison	Granulation of mash			Diff. req. for sig. P = 0.05
	Coarse	Medium	Fine	
Number of birds at start at experiment	90.0	90.0	90.0	—
Number of birds at end of experiment	80.0	80.0	77.0	—
Per cent mortality	11.1	11.1	14.4	—
Egg production—				
Av. on a hen-house basis	118.0 ± 55.0 <sup>2</sup>	125.0 ± 49.0	113.0 ± 48.0	—
Av. on a survival basis	132.0 ± 41.0	137.0 ± 34.0	125.0 ± 34.0	—
Av. pounds of eggs per bird	17.8 ± 5.5	18.5 ± 4.5	16.9 ± 4.6	—
Feed consumption (pounds) <sup>3</sup> —				
Av. feed cons. per bird	84.3 ± 10.1	86.9 ± 9.6	81.2 ± 9.2	3.0
Av. feed cons. per 100 birds per day	30.1 ± 2.8	31.0 ± 2.7	29.0 ± 2.6	1.1
Av. feed cons. per bird (adjusted) <sup>3</sup>	84.5	85.9	82.0	1.8
Feed efficiency for egg production—				
Number of eggs per 100 pounds of feed	155.0 ± 41.0	157.0 ± 34.0	154.0 ± 37.0	—
Pounds of eggs per 100 pounds of feed	20.9 ± 5.5	21.2 ± 4.4	20.7 ± 4.8	—
Body weight of birds (pounds)—				
Av. initial body weight	5.18 ± 0.53	5.29 ± 0.57	5.30 ± 0.70	—
Av. final body weight	6.50 ± 0.98	6.57 ± 0.98	6.56 ± 1.07	—
Av. individual egg weight (gm.)	61.4 ± 3.4	61.4 ± 3.9	61.6 ± 3.2	—
Av. specific gravity of eggs	1.0803 ± 0.0043	1.0809 ± 0.0037	1.0805 ± 0.0037	—

<sup>1</sup> Duration of experiment, 280 days.<sup>2</sup> Standard deviation.<sup>3</sup> Feed consumption adjusted for differences in body weight and egg production.

However, Berg and Bearse (2) have shown that birds will adapt themselves quite rapidly to changes of granulation in the mash without any serious effect on egg production. The work of Berg and Bearse is not directly comparable with the work reported herein since they fed a grain and mash ration. Hence any advantage which might result from a saving of energy in the grinding or increased digestibility of fine particles would not be reflected in the results of their work since forty per cent of the ration was whole grains.

While the preference for a coarse feed may have had some slight effect on the lower feed consumption of the birds on the fine ration, a lack of any significant difference in egg production indicates that the inherent ability of the birds to produce was not affected by the lower feed consumption. The results of these experiments show that laying hens will readily consume and efficiently utilize rations varying in texture. Feed efficiency shows some advantage for the fine rations. The only apparent explanation for this is the possibility that the birds on the fine ration, being denied the



opportunity to pick and choose, get a more balanced ration, or that the finer feed is more readily digested and assimilated by the bird, resulting in improved feed efficiency.

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# GERMINATION OF WEED SEEDS

## III. THE INFLUENCE OF CROPS AND FALLOW ON THE WEED SEED POPULATION OF THE SOIL<sup>1</sup>

A. C. BUDD<sup>2</sup>, W. S. CHEPIL<sup>3</sup>, AND J. L. DOUGHTY<sup>4</sup>

*Soil Research Laboratory, Swift Current, Sask.*

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### ABSTRACT

Information is presented showing the relative influence of crops and fallow on the number of annual weed seeds in the soil under the semi-arid conditions of southwestern Saskatchewan. Soil samples were collected in the spring and fall from each strip of a field in a 3-year rotation of fallow - wheat - wheat. The number of viable seeds was determined by germination tests in the laboratory. Sampling was continued for 6 years, the same locations being sampled each time. The data show a reduction in total viable seeds from spring to fall when the land was fallowed. There was an increase in viable seeds from spring to fall in the cropped land each year, except one. In general, there was a reduction in viable seeds during the over winter period. Summerfallowing was most effective in controlling weeds whose seeds have a short period of dormancy. As climatic conditions influenced weed development, the weed seed populations varied in kind and number from year to year. At all times during the experiment there were sufficient weed seeds in the soil to produce a dense weed cover.

### INTRODUCTION

The persistence of annual weeds is dependent mainly on their ability to re-infest the soil with viable seeds. In order that crops may be grown successfully, it is necessary that the cropping and tillage methods have the greatest possible effect in reducing the weed seed population of the soil. This paper gives information on the relative influence of crops and fallow on the population of seeds of different species.

<sup>1</sup> Contribution from the Division of Field Husbandry, Soils and Agricultural Engineering, Experimental Farms Service, Department of Agriculture, Canada.

<sup>2</sup> Now Range Botanist, Experimental Station, Swift Current, Sask.

<sup>3</sup> Now Department of Soils, State College, Manhattan, Kansas.

<sup>4</sup> Officer-in-Charge.

TABLE 1.—FIELD ROTATION

Year	Strip Y - 6	Strip Y - 5	Strip Y - 4
1937	Fallow	—	—
1938	Wheat	Fallow	—
1939	Wheat	Wheat	Fallow
1940	Fallow	Wheat	Wheat
1941	Wheat	Fallow	Wheat
1942	Wheat	Wheat	Fallow
1943	—	Oats	Oats
1944	—	—	Wheat



The general cropping practice in the semi-arid region of southwestern Saskatchewan includes one year of fallow followed by one or two grain crops. The main purpose of the fallow is to conserve soil moisture, although in certain areas the control of weeds is of primary importance. Cultural operations are planned for the maximum weed control with the minimum pulverization of the soil and burial of crop residue thus reducing the danger of erosion. Surface tillage implements are used in these operations thus giving rise to the term 'ploughless fallow'.

#### METHODS OF PROCEDURE

Soil samples from each field strip in a 3-year rotation of fallow – wheat – wheat (Table 1), were taken early in the spring before any seeds germinated and just before freeze-up in the fall. Each strip, starting with the fallow year, was sampled twice yearly for 6 years.

The samples were taken with a rectangular tube  $3 \times 4$  inches in dimension which was pressed into the soil to a depth of 6 inches and the enclosed soil scooped out. Twenty-four samples were collected on each strip at each sampling date and at approximately the same locations for the duration of the experiment (Figure 1).

The samples from each strip were bulked and washed through an 80-mesh sieve, which prevented the loss of even the smallest seeds. The weed seeds and the residue were placed in saucers and subjected to repeated germination tests in the laboratory. The time required for all the seeds to germinate would be at least 5 years, although approximately 75 per cent



FIGURE 1. Sampling outfit. Note proximity of spring and fall sampling sites.

TABLE 2.—NUMBER OF VIABLE WEED SEEDS PER SQUARE FOOT TO 6-INCH DEPTH IN A FALLOW, WHEAT, WHEAT ROTATION

Plot Y-6												
Weed species	Fallow, 1937		Wheat, 1938		Wheat, 1939		Fallow, 1940		Wheat, 1941		Wheat, 1942	
	April 14	Oct. 20	April 14	Oct. 20	April 13	Oct. 19	April 22	Oct. 18	April 12	Oct. 21	April 14	Oct. 30
Stinkweed	839	565	580	681	545	1638	1288	226	226	530	500	666
Russian thistle	134	19	22	234	426	162	207	88	40	406	250	155
Lamb's quarters and amaranth	137	150	136	27	78	94	84	53	96	140	84	92
Tumbling mustard	50	129	61	24	83	234	182	41	34	61	42	32
Other weeds	31	4	1	2	4	3	2	1	4	2	6	4
Total	1196	874	800	968	1136	2131	1763	409	400	1139	882	949

Plot Y-5												
Weed species	Fallow, 1938		Wheat, 1939		Wheat, 1940		Fallow, 1941		Wheat, 1942		Oats, 1943	
	April 14	Oct. 20	April 13	Oct. 19	April 22	Oct. 18	April 12	Oct. 21	April 14	Oct. 30	April 14	Oct. 28
Stinkweed	454	253	295	644	434	310	307	256	244	436	362	432
Russian thistle	37	21	28	8	31	19	18	14	6	10	6	238
Lamb's quarters and amaranth	124	43	92	126	126	94	136	71	120	138	102	100
Tumbling mustard	19	1	9	23	23	17	20	17	5	6	15	26
Other weeds	0	0	1	1	2	2	2	2	2	1	1	2
Total	634	318	426	802	616	442	479	360	377	591	486	798

Plot Y-4												
Weed species	Fallow, 1939		Wheat, 1940		Wheat, 1941		Fallow, 1942		Oats, 1943		Wheat, 1944	
	April 13	Oct. 19	April 22	Oct. 18	April 12	Oct. 21	April 14	Oct. 30	April 14	Oct. 28	April 14	Oct. 26
Stinkweed	250	476	279	186	174	224	202	139	92	748	678	659
Russian thistle	560	223	392	204	152	126	85	28	20	458	370	624
Lamb's quarters and amaranth	86	79	82	94	94	72	78	60	20	86	94	46
Tumbling mustard	11	18	12	22	22	18	10	16	26	13	23	12
Other weeds	3	0	1	1	0	1	1	0	2	2	2	1
Total	911	796	766	507	442	441	376	243	223	1307	1167	1342



of the viable seeds germinated within the first year and 90 per cent within the first 3 years. The seedlings were identified, counted, and recorded as soon as they emerged. Determinations were made, not of the total number of seeds present in the soil, but of the number of seeds that germinated. Seedlings of *Chenopodium album* and *Amaranthus spp.* could not be identified at an early stage and were, therefore, grouped together. No difficulty was experienced in identifying the other species.

### RESULTS

Table 2 gives a summary of the variations in the number of viable seeds in the soil as influenced by crops and fallow during the period 1937-1944, inclusive. These data are based on the numbers of seedlings emerged under germination tests in the laboratory up to February 15, 1946. Any seeds that might have germinated after this date would have had little influence on the final results.

The following species were found, in order of relative abundance of viable seeds in the soil: *Thlaspi arvense* (stinkweed), *Salsola pestifer* (Russian thistle), *Sisymbrium altissimum* (tumbling mustard), *Chenopodium album* (lamb's quarters), *Amaranthus retroflexus* (red-root pigweed), *A. albus* (tumbleweed), *A. graecizans* (prostrate amaranth), *Descurainia Sophia* (flixweed), *Polygonum Convolvulus* (wild buckwheat) and *Lepidium densiflorum* (wild peppergrass). The number of viable seeds of the latter three was collectively very low. For convenience, the weeds will be referred to by their common names.

The results show a wide variation in weed seed population (Table 2) between species and from year to year. Some of the yearly variations were due to climatic conditions.

For convenience in interpreting the data, the results for individual species are tabulated on a percentage basis, designating the number of viable seeds present in April at the start of the first fallow period as 100 and recording the number found at subsequent dates as a percentage of the original number (Table 3).

### INFLUENCE OF PLOUGHLESS FALLOW ON WEED SEED POPULATION

#### *Stinkweed*

In all years, except 1939, the ploughless fallow reduced the numbers of viable stinkweed seeds. Continued wet weather during the latter half of May and beginning of June in 1939 made it impossible to work the land until after June 28 and by that time many plants were well past the flowering stage and some had produced viable seeds. None of the other weeds reported in this investigation matured as rapidly as stinkweed and all were destroyed by tillage before seed formation.

The greatest effect of the fallow on stinkweed seeds occurred in the relatively dry season of 1940 when the original population was reduced by more than 80 per cent. The smallest reduction, which amounted to only 17 per cent, was in 1941, another relatively dry season. The stinkweed is a prolific plant, producing seeds which shatter easily and may remain dormant for several years (1). For these reasons it builds up a large reserve of seeds in the soil. At no time during the 6 years was the population of stinkweed seeds reduced to negligible numbers, but was far greater than that required to produce a heavy growth of weeds.

TABLE 3.—CHANGE IN WEED SEED POPULATION. PERCENTAGE BASIS

Plot Y-6													
Weed species	Fallow, 1937		Wheat, 1938		Wheat, 1939		Fallow, 1940		Wheat, 1941		Wheat, 1942		
	Original No.	April	October	April	October	April	October	April	October	April	October		
Stinkweed	839	100	67	69	81	65	154	27	63	60	79		
Russian thistle	134	100	14	16	175	318	154	30	303	187	116		
Lamb's quarters and amaranth	137	100	109	100	20	57	61	39	102	61	67		
Tumbling mustard	50	100	258	122	48	166	364	82	122	84	64		
Other weeds	31	100	13	3	6	13	6	3	6	19	13		
Total	1196	100	73	67	81	95	147	34	95	74	79		

Plot Y-5													
Weed species	Fallow, 1938		Wheat, 1939		Wheat, 1940		Fallow, 1941		Wheat, 1942		Oats, 1943		
	Original No.	April	October	April	October	April	October	April	October	April	October		
Stinkweed	454	100	56	65	142	96	68	56	96	80	95		
Russian thistle	37	100	57	76	22	84	51	38	27	16	643		
Lamb's quarters and amaranth	124	100	35	74	102	102	76	110	111	82	81		
Tumbling mustard	19	100	5	47	121	121	89	105	32	79	137		
Other weeds	(1939) 1	0	0	100	100	200	200	200	100	100	200		
Total	634	100	50	67	126	97	70	57	93	77	126		

Plot Y-4													
Weed species	Fallow, 1939		Wheat, 1940		Wheat, 1941		Fallow, 1942		Oats, 1943		Wheat, 1944		
	Original No.	April	October	April	October	April	October	April	October	April	October		
Stinkweed	250	100	190	112	74	70	80	56	299	271	264		
Russian thistle	560	100	40	70	36	27	15	5	82	66	111		
Lamb's quarters and amaranth	86	100	92	95	109	109	91	70	96	109	53		
Tumbling mustard	11	100	164	109	200	200	91	145	118	209	109		
Other weeds	3	100	0	33	33	0	33	0	66	66	33		
Total	911	100	87	84	56	48	41	27	143	128	147		



*Russian Thistle*

The seeds of this plant have a short period of natural dormancy and are, therefore, much better controlled by fallowing than most other species. Although the reductions in viable seeds under fallow were high, they were not as great as those obtained in other experiments with fallow (1, 2), in which all seeds were in contact with the soil. The reduction in the numbers by the fallow would have been much greater had all the seeds been scattered on the surface of the ground or buried by tillage. Many seeds were held by the old plants which were left unburied to serve as protection against wind erosion. All weeds studied in this investigation, except the Russian thistle, shatter their seeds readily at maturity and are, therefore, not similarly affected. Russian thistle makes considerable growth late in the season and generally does not produce any seeds until after the end of August. Late tillage was avoided because of the hazard of soil drifting, which allowed some plants that emerged late to reach maturity and add fresh seeds to the soil.

*Lamb's Quarters and Amaranth*

The average reduction in the numbers of seeds of this group by fallowing was about 30 per cent. Owing to the similar appearance of the seedlings of this group at an early stage no attempt was made to separate the different species. The results obtained in another experiment (1) show that these four species have an approximately equal dormancy period; hence the results obtained with this group as a whole would apply more or less to each individual species. The fallow was somewhat less effective in reducing the seed population of these species than of stinkweed and much less than of Russian thistle. The experimental error is large when the seed population is very low.

*Tumbling Mustard*

The effect of the fallow on the population of tumbling mustard seeds was erratic, possibly on account of the relatively small numbers present in the soil. In 4 out of 6 years the seed population in the soil was negligible and only on two occasions were more than 100 seeds per square foot found. This weed has a very long period of seed dormancy (1) and the seeds in the soil were consequently little affected by fallowing.

**THE INFLUENCE OF GRAIN CROPS ON THE WEED SEED POPULATION**

The usual procedure in this area is to work the land in the spring and to seed at the same time or within a few days. Following these operations both the grain and weeds emerge when conditions are favourable for germination. If conditions are such as to favour a rapid development of the grain, the weeds may be suppressed and a clean crop result. If, on the other hand, the early growth of grain is poor, weeds will flourish, and restock the land with large numbers of seeds. The relative abundance of each species under conditions of poor crop growth is dependent mainly on seasonal conditions. As the conditions vary from year to year, the competitive balance between different weeds and the crop varies also, and in no two consecutive years can the weed population be expected to be of the same general composition.



FIGURE 2. Wheat stubble heavily infested with Russian thistle in dry year of 1938.

The total weed seed population at the beginning and end of the first and second grain crops after fallow are recorded in Table 2. The relative percentage of seeds for the same periods based on the numbers originally present in the fallow at the start of the experiment are given in Table 3. The number of weed seeds varied widely from year to year but in general showed an increase in the crop years except for 1940. The maximum decrease in any one year was 80 per cent for lamb's quarters and amaranth in 1938 and the maximum increase was 627 per cent for Russian thistle in the second year crop of 1943. These wide variations were attributed to the variable effect of the seasons, for climatic conditions favouring the development of some species are often unfavourable for others.

#### *Stinkweed*

The seed population increased materially in both the first and the second grain crops after fallow, this occurring every year except the dry year of 1940, when slight decreases in both fields were noted. On the whole, stinkweed populations increased more in the first crop after fallow than in the second. The greatest increase, 262 per cent, occurred in the first crop after fallow in 1943 and the population continued high throughout the following year.

#### *Russian Thistle*

The populations were subject to very marked changes from year to year. The infestations increased markedly in the relatively dry seasons of 1938, 1941 and 1943 and decreased in the relatively wet season of 1939.





FIGURE 3. Wheat stubble free of Russian thistle in relatively wet year of 1939.

Substantial decreases were encountered in both the first and the second crops after fallow in 1940, a relatively dry season (Figures 2 and 3).

The second stubble after fallow was cultivated with a blade weeder soon after harvest in 1941, resulting in virtually complete destruction of a heavy growth of immature Russian thistles, which would have ripened before the end of the growing season and re-stocked the land with viable seeds. The blade weeding had no effect on the populations of other species which had reached maturity at the time of the treatment.

#### *Lamb's Quarters and Amaranth*

These species were little affected by either the first or the second crop after fallow and seemed to survive equally well under all types of season.

#### *Tumbling Mustard*

The numbers of viable seeds of this species were too low to permit an accurate determination of the influence of tillage and cropping methods on the seed population.

The data show, in general, appreciable increases in weed seeds in both the first and second crops after fallow, but such increases were reduced by mortality of the seeds during the late fall, winter and early spring.

#### CHANGES IN THE WEED SEED POPULATION DURING LATE FALL, WINTER, AND EARLY SPRING

There was practically no vegetative growth between the October and April samplings except for a week or two at the beginning and the end of this period as the land was frozen almost continually. Weed seedlings





FIGURE 4. Russian thistle caught by fence after being blown across fallow.

made their first appearance about April 30, although the actual germination of seeds in some years began as early as the first week in April. The germinating seeds occasionally found in April samplings were almost always stinkweed or Russian thistle. Newly germinated seeds were seldom found in the October samplings. When seedlings were found in the soil at the time of the sampling they were picked out, identified, and recorded as part of the population.

The changes in population, if any, occurring during the winter period may be due to one or more of the following causes:

(1) Increase or decrease in population resulting from seeds being blown in or out by the wind. The movement of weed seeds takes place in two general ways, by whole plants being broken off at maturity and blown along the surface of the ground, shattering seeds as they go, and/or seeds scattered on the ground being blown along with drifting soil or snow. Russian thistle (Figure 4) is particularly susceptible to transport by the former method and lamb's quarters and amaranth by the latter. Tumbling mustard seeds are readily transported by the wind.

(2) The loss of vitality by dormant seeds.

(3) Decrease in population due to partial germination and death of seeds of low vitality. Russian thistle seeds are particularly susceptible to attack and destruction by moulds and fungi during germination.

The data show that stinkweed was the major species most consistently reduced in numbers during the late fall, winter and early spring. Since there was little or no removal nor deposition of seeds, the decrease must

be attributed to the loss of seed vitality or the partial germination and death of seeds under an environment that was adverse to growth.

The number of viable seeds of all other species varied widely for the winter period. These variations are to be expected in view of the various factors involved.

### DISCUSSION

Summerfallow, as a method for the eradication of weeds, is far more effective on those whose seeds have a short dormancy, such as Russian thistle.

The prevalence of different species in the soil is attributed to climatic conditions which vary from year to year thus causing changes in the relative populations.

The general weed flora is made up of species, the growth and development of which does not materially interfere with the growth and development of the other component species.

### ACKNOWLEDGMENT

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# SEED PRODUCTION STUDIES WITH RUSSIAN WILD-RYE<sup>1</sup>

H. B. STELFOX<sup>2</sup>, D. H. HEINRICHS<sup>3</sup>, AND R. P. KNOWLES<sup>4</sup>

*Experimental Farms Service, Canada Department of Agriculture*

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## ABSTRACT

Studies with Russian wild-rye were conducted at Saskatoon and Swift Current, Saskatchewan, and Lacombe, Alberta, to determine the effect on seed production of row spacing, fertilizer treatments, association with alfalfa, and early spring burning. Row spacing was found to be the most important factor influencing seed yield. Optimum spacing for rows varied from two to four feet, depending upon moisture conditions and age of stand. Fertilizer responses were obtained only in years of adequate moisture. Ammonium phosphate 16-20 at a rate supplying approximately 40 pounds of nitrogen per acre was more beneficial in the second and subsequent crop years at Swift Current and Lacombe than one-half that rate. At Saskatoon, nitrogen at 84 pounds per acre produced higher yields than 42 pounds. The residual effect of fertilizer was quite pronounced at Saskatoon. Alfalfa grown in association with Russian wild-rye slightly reduced seed yields of the grass. Early spring burning increased seed yield in some instances and had no effect in others.

## INTRODUCTION

Russian wild-rye (*Elymus junceus* Fisch.) is showing considerable promise as a dryland pasture species for Western Canada. However, early experience with seed production of this grass has been disappointing. Seed yields fluctuate greatly from year to year and stands frequently fail to produce seed. In 1947 preliminary tests were started at several points in Western Canada to investigate the effect on seed yield of row spacing, commercial fertilizer, and removal of aftermath growth by burning. This paper presents results of the tests conducted at the Experimental Stations at Swift Current, Saskatchewan, and Lacombe, Alberta, and the Forage Crops Laboratory at Saskatoon, Saskatchewan.

The beneficial effect of row seeding on seed yields of several grasses has been reported by Klages and Stark (6) and Cornelius (2). Different species were found to vary in their response to row spacing. Soil fertility, age of stand and moisture conditions also influenced the results obtained. Specific recommendations for row spacing of Russian wild-rye have been made by Rogler (10) and Irwin (5).

While no published data are available on the effect of fertilizer on seed yields of Russian wild-rye, numerous studies with other grasses have been reported (3, 4, 7, 8). Generally, the use of nitrogen fertilizers has resulted in increased yields but only in occasional instances have phosphate and potash fertilizers been beneficial. Rate of application and, to a lesser extent, source of nitrogen have influenced the effect of fertilizer. Soil fertility, age of stand and moisture conditions have been important factors also. Marked residual effects of nitrogen on brome grass were noted by Knowles and Cooke (7). The same authors found August and September applications of nitrogen to be more satisfactory than late fall or early spring treatments.

<sup>1</sup> Contribution from Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada.

<sup>2</sup> Agricultural Research Officer, Experimental Station, Lacombe, Alta.

<sup>3</sup> Agricultural Research Officer, Experimental Station, Swift Current, Sask.

<sup>4</sup> Agricultural Research Officer, Forage Crops Laboratory, Saskatoon, Sask.



Removal of aftermath growth by burning has given variable response in seed production of grasses (1, 2). Some species were found to respond favourably to burning, while with others it was detrimental. Where disease infection was severe on creeping red fescue, Musser (9) found that summer burning following seed harvest materially increased the seed yield the following year while spring burning had no effect.

### MATERIALS AND METHODS

The tests at Saskatoon, Swift Current and Lacombe were conducted under quite widely different soil and climatic conditions. At Saskatoon the tests were on a silty clay loam soil of the dark brown soil zone, at Swift Current on a clay loam soil of the brown soil zone, and at Lacombe on a sandy loam soil of the black soil zone. Precipitation records for the three different locations for the period 1947 to 1952 are given in Table 1; also the evaporation records for Saskatoon and Swift Current for that period.

The seed of Russian wild-rye used for establishing the tests was obtained from a bulk lot originating from an introduction of this grass to the University of Saskatchewan in 1927 from the Western Siberian Experiment Station at Omsk. A botanical description and the growth characteristics of Russian wild-rye are given by Rogler (10).

The tests at Swift Current and Lacombe were of the split plot design. The main split divided each replicate in two and compared the effect of early spring burning versus non-burning of the aftermath growth. The second split divided each replicate into four plots comparing the effect of spacing rows 1, 2, 3 and 4 feet apart. The plot sequence was the same in the burned as in the non-burned plot in each replicate. Each spacing plot

TABLE 1.—PRECIPITATION DURING THE CROP YEAR PERIOD AUGUST TO JULY, INCLUSIVE, AND THE EVAPORATION DURING AUGUST, SEPTEMBER, MAY, JUNE AND JULY OF EACH CROP YEAR FOR THE YEARS OF THE EXPERIMENTS REPORTED\*

Crop year	Precipitation in inches			Evaporation from free water surface in inches	
	Saskatoon	Swift Current	Lacombe	Saskatoon	Swift Current
1946-47	12.39	13.49	19.03	23.09	28.78
1947-48	15.32	14.98	27.35	21.91	26.66
1948-49	12.74	9.05	13.99	22.37	33.81
1949-50	16.45	15.75	14.10	19.33	30.13
1950-51	16.32	15.83	19.90	17.81	25.52
1951-52	13.67	19.94	13.69	17.99	22.35
Mean	14.48	14.84	18.84	20.42	27.87

\* Data for Saskatoon and Swift Current supplied by the Physics Department, University of Saskatchewan, Saskatoon, and the Soils Research Laboratory, Swift Current, Sask., respectively.

was subdivided for the following comparisons: (1) no fertilizer; (2) ammonium phosphate 16-20 at 135 pounds per acre; (3) ammonium phosphate at 250 pounds per acre, and (4) grass seeded with alfalfa at one pound per acre. The sub-plots were 40 feet long. The sub-sub-plots for the 2-, 3- and 4-foot spacings consisted of three rows, while the plots for the 1-foot spacing consisted of six rows.

The tests were seeded on summerfallow land without a companion crop. The rate of seeding was 7 pounds per acre for rows spaced 1 foot apart. Fertilizer treatments at Swift Current were applied broadcast late in the fall each year, except in 1952 when they were applied early in the spring. At Lacombe the fertilizer was applied with the seed at the time of seeding. Later applications were broadcast early in the spring of 1948, in mid-October of 1948 and 1949 and in mid-August of 1950 and 1951.

Burning was done on the Lacombe test with a home-made coal-oil torch while at Swift Current a flame-thrower was used. The spring burning treatments at Lacombe were not applied in 1948 or 1952 because of insufficient growth. Since no response to burning was obtained in the three intervening years the yields from the burned and unburned plots were bulked and the burning treatment ignored in the analysis.

The centre two rows of each sub-sub-plot were harvested by hand in the case of rows spaced 1 foot apart and only the centre row was harvested for the wider spaced rows. The Swift Current test produced no seed in 1948 because of poor plant development in the seedling year. At Lacombe the sub-sub-plots containing alfalfa were contaminated with timothy which appeared to have a depressing effect on the seed production of Russian wild-rye; consequently, yields were not harvested from these plots.

At Saskatoon a series of trials involving nitrogen fertilizers and a burning treatment were conducted from 1947 to 1952 on a seed increase block seeded in 1945 in rows spaced 3 feet apart. A randomized block design with three replicates was used. Each plot ran across 13 rows and occupied an area of approximately 10 feet by 40 feet. Fertilizer treatments were broadcast early in the spring. A 3-foot width of cut was harvested for yield determinations from 11 rows of each plot. Practically no seed was produced in 1949 and the test was not harvested that year.

A further test involving burning, fertilizers, row spacing and plant spacing was established at Saskatoon in 1947. A randomized block design with four replicates was used. Three spacing treatments were involved: (1) rows 3 feet apart; (2) rows 3 feet apart with plants 2 feet apart within the rows; and (3) rows 1 foot apart. In addition to the spacing comparisons, one manure and two fertilizer treatments applied to rows spaced 1 foot apart were also included. Plots were 6 feet by 16 feet for rows spaced 1 foot apart, and 12 feet by 16 feet for rows 3 feet apart. Two replicates were seeded on summerfallow and the other two on stubble land. Manure and fertilizer treatments were applied broadcast early in the spring of 1948, 1949, and 1950. The three centre rows were harvested for yield determinations from the plots of 1-foot row spacing while the single centre row was harvested for rows spaced 3 feet apart. This test also failed to produce any appreciable amount of seed in 1949 and was not harvested that year.

## RESULTS

*Swift Current and Lacombe*

The results obtained from various treatments are given in Tables 2 and 3.

TABLE 2.—EFFECT OF ROW SPACING AND MANAGEMENT PRACTICES ON THE SEED YIELDS OF RUSSIAN WILD-RYE — SWIFT CURRENT, 1949–1952

Comparison	Average seed yield – lb. per acre				
	1949	1950	1951	1952	Mean
1-foot row spacing	3	34	42	117	49
2-foot row spacing	42	139	156	207	136
3-foot row spacing	60	181	209	195	161
4-foot row spacing	67	210	262	199	185
L.S.D. (P = 0.05)	31	57	49	N.S.	22
Check – no fertilizer	47	137	151	111	112
135 lb./ac. A.P. 16-20-0	51	157	188	195	148
250 lb./ac. A.P. 16-20-0	52	167	210	293	180
Seeded with alfalfa	23	102	121	119	91
L.S.D. (P = 0.05)	N.S.	34	65	37	17
Burned	49	158	177	183	142
Not burned	37	124	158	176	124
L.S.D. (P = 0.05)	12	N.S.	18	N.S.	11
Average all treatments	43	141	167	179	133

TABLE 3.—EFFECT OF ROW SPACING AND FERTILIZER ON THE SEED YIELDS OF RUSSIAN WILD-RYE — LACOMBE, 1948–1952

Comparison	Average seed yield – lb. per acre					
	1948	1949	1950	1951	1952	Mean
1-foot row spacing	344	400	245	181	61	246
2-foot row spacing	181	880	463	295	143	392
3-foot row spacing	92	744	510	343	183	374
4-foot row spacing	87	598	517	368	181	350
L.S.D. (P = 0.05)	61	144	60	82	67	46
Check – no fertilizer	96	678	418	264	131	318
100 lb./ac. A.P. 16-20-0	211	638	455	299	146	350
225 lb./ac. A.P. 16-20-0	220	650	428	328	148	355
L.S.D. (P = 0.05)	27	N.S.	N.S.	29	N.S.	28
Average all treatments	176	656	434	297	142	341



The data show that the highest yields were obtained at Swift Current from the 4-foot plantings, while at Lacombe, located in a moister climate, the 2-foot and 3-foot plantings gave the best yields. The application of ammonium phosphate 16-20 fertilizer gave a significant increase in seed yield at both Lacombe and Swift Current. The heavier application of 250 pounds per acre gave a significant increase over the 135-pound rate at Swift Current but not at Lacombe. The presence of alfalfa in the stand of Russian wild-rye depressed the seed yield significantly.

The wider the row spacing, the greater was the seed yield at Swift Current every year except in 1952 when there was no difference between the yields from rows spaced one and two feet apart. At Lacombe the yield differential between various spacings was not nearly so marked as at Swift Current as the age of the stand progressed. In dry years (Table 1) wider spacings gave a proportionally greater yield increase than in wet years. Analysis of variance shows a highly significant spacing  $\times$  year interaction for both tests.

The yield response to fertilizer at Swift Current appeared to increase with the age of the stand while at Lacombe the response seemed to vary from year to year. Significant effects of fertilizer at Lacombe were noted only in 1948 and 1951 when moisture conditions were very favourable. No response to fertilizer was obtained at Swift Current in 1949 when precipitation was very light. The fertilizer  $\times$  year interaction was highly significant at both stations.

The effect of burning gave a slight increase in yield every year, although this increase was statistically significant only in 1949 and 1951.

### *Saskatoon*

The results obtained from nitrogen fertilization and spring burning of a row seeding are given in Table 4.

TABLE 4.—EFFECT OF FERTILIZER AND BURNING TREATMENTS ON SEED YIELDS OF RUSSIAN WILD-RYE GROWN IN ROWS THREE FEET APART — SASKATOON

Treatment	Rate of fertilization*	Time of application	Seed yield — lb. per acre			
			1947	1948	1950	1951
Check	—	—	29	121	41	26
Am. sulph.	42**	Spring 1947, 1949, 1950	22	189	25	46
Am. sulph.	84	Spring 1947, 1949, 1950	26	232	69	88
Am. sulph.	42	Spring 1948, 1949, 1950	—	94	37	36
Am. sulph.	84	Spring 1948, 1949	—	93	52	10
Burning	—	Spring 1948, 1950	—	96	45	33
L.S.D. (P = 0.05)		—	N.S.	46	21	32

\* Pounds of elemental nitrogen per acre.

\*\* Eighty-four pounds of nitrogen applied as ammonium nitrate in the spring of 1950.

TABLE 5.—EFFECT OF ROW AND PLANT SPACING, FERTILIZER AND BURNING TREATMENTS ON SEED YIELDS OF RUSSIAN WILD-RYE—SASKATOON. TREATMENTS APPLIED EARLY IN SPRING OF 1948, 1949 AND 1950

Spacing and treatment	Seed yield – lb. per acre			
	1948	1950	1951	3-yr. av.
Rows 3 feet apart	46	209	128	128
Rows 3 feet apart – plants spaced 2 feet apart	139	308	153	200
Rows 1 foot apart	114	131	57	101
Rows 1 foot apart – 300 lb./ac. am. sulph.	55	142	44	80
Rows 1 foot apart – 188 lb./ac. am. nit.*	47	132	52	77
Rows 1 foot apart – 6 tons/ac. manure	90	78	43	70
Rows 1 foot apart – aftermath growth burned	—	170	96	—
L.S.D. (P = 0.05)	N.S.	106	42	—

\* Four hundred pounds per acre of ammonium phosphate 16-20 applied in 1948 instead of ammonium nitrate.

The data show little or no direct increase in yield for nitrogen but a pronounced residual effect is evident, particularly for the 84-pound rate in 1948 and 1951. From this seeding additional data were obtained in 1952 on the effect of nitrogen at 21, 42 and 84 pounds per acre applied September 5, 1951 and for 42 pounds per acre applied October 2 and November 7, 1951 and April 4, 1952. Seed yields in 1952 averaged only 24 pounds per acre and none of the treatments gave a significant yield increase although an increase trend was evident for the 84-pound rate. Unfavourable moisture conditions may account for the failure of the seed crop in 1949 and the lack of response to nitrogen fertilizer in 1947 and 1952. Burning had no effect on seed yield.

The results obtained from a further test involving various spacing, fertilizer and burning treatments are given in Table 5. Spacing rows 3 feet apart, as compared with 1-foot spacing, resulted in higher seed yields in the third and fourth crop years. Spacing plants 2 feet apart within the wider-spaced rows did not give a significant yield increase, but an increase trend is indicated. A yield increase trend is also indicated for the effect of early spring burning of the grass grown in rows 1 foot apart, but fertilizer treatments did not result in a yield increase. Nitrogen applied at 84 pounds per acre in late summer, early fall and late fall in 1951 and early in the spring in 1952, to the plots having 1-foot row spacing, likewise failed to increase the seed yield.

#### DISCUSSION

Satisfactory seed yields of Russian wild-rye were obtained in most instances when suitable management practices were followed. Type of seeding, stand establishment, soil fertility and moisture supply were the most important factors determining seed yield.

Row spacing was the most important single factor affecting seed production. Four-foot spacing produced the highest yields under limited moisture at Swift Current, while 2-foot spacing was most productive at Lacombe where moisture conditions were more favourable. At Saskatoon, 3-foot spacing was superior to 1-foot spacing. The optimum row spacing was found to vary with the age of the stand as well as with moisture conditions. In general, rows 3 to 4 feet apart were more productive than narrower-spaced rows after the second crop year. The spacing of plants within the rows also seemed to be advantageous under dry conditions.

A favourable moisture supply appeared to be important for successful seed production. Seed yields were very light at Saskatoon in 1947 and negligible in 1949 when precipitation was light and evaporation high. At Swift Current, 1949 was dry also and the seed yield was low. Although the precipitation at Lacombe was below average for the crop years 1948-49 and 1949-50 the moisture supply apparently was adequate for successful seed production. Seasonal distribution of rainfall also may have been important in determining seed yield. Russian wild-rye produces culms early in the spring; consequently, the amount of late fall and early spring moisture may govern culm production to a large extent.

The effect of good stand establishment was very apparent from the test at Saskatoon in which two replicates were seeded on summerfallow and the other two on stubble land. In the first crop year the average yield on the summerfallow was 138 pounds per acre, compared to only 27 pounds per acre on the stubble land. No seed was produced on the Swift Current test in 1948 because of poor plant development in the seedling year. The yield in the first crop year at Lacombe was much lower than yields for the second and third crops. Companion crop seedings and late spring and summer seedings without a companion crop usually have produced little or no seed the year after seeding.

Fertilizer was less important than row spacing in influencing seed yield. Fertilizer treatments applied to rows 1 foot apart did not compensate for the beneficial effect of wider spacing as the stands became older. Favourable responses to nitrogen and nitrogen plus phosphorus were obtained only in years of good moisture. The Saskatoon results suggest that, in addition to moisture, the time of application of fertilizer might have been an important factor. Most of the yield increases due to fertilizer at Saskatoon resulted from treatments applied the previous year. Since fertilizer treatments at Swift Current and Lacombe were applied annually, yield increases from fertilizer after the first crop year may have been due, in part at least, to a residual effect of the fertilizer. A critical comparison of rates of nitrogen was not possible from the studies reported. Similarly, the value of applying phosphorus with nitrogen could not be ascertained. The heavier rates of fertilizer, however, were the most effective as the age of the stands increased. Ammonium phosphate 16-20 at a rate supplying approximately 40 pounds of nitrogen per acre was more beneficial in the second and subsequent crop years at Swift Current and Lacombe than one-half that rate. At Saskatoon, nitrogen at 84 pounds per acre produced higher yields than the 42-pound rate.



Growing Russian wild-rye for seed in association with a legume such as alfalfa did not benefit seed yield and it reduced yields significantly at Swift Current in 1950.

Early spring burning of stubble and aftermath growth was not harmful to seed production and in some instances it was beneficial. Burning significantly increased seed yields at Swift Current in two years out of four, while at Saskatoon a yield increase trend resulted from burning treatments applied to a row seeding of 1-foot spacing. Further investigations are required to determine whether removal of growth by late fall clipping or grazing will produce results similar to early spring burning. Experiments are now under way at Swift Current and Lacombe to study the effect of various degrees of utilization of aftermath growth on subsequent seed yield and to compare the effect of rates, formulations and times of application of fertilizer.

An extensive seed multiplication program launched by the Swift Current Station in 1950 is ensuring a rapid increase in seed supplies of Russian wild-rye in Western Canada within the next few years. It is also providing additional information on seed production problems associated with this grass. Harvesting the seed is presenting a problem since it shatters readily. When grown in rows, as it must be grown, it is difficult to pick up when swathed and if straight combined much seed shatters. Most of the growers favour straight combining on the green side and drying the seed. The seed threshes out easily even if green; also it comes out of the combine quite clean as the straw does not break up to any extent. More investigation in harvesting methods is required.

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# "FEED-ANI" AS A RATION COMPONENT FOR POULTRY AND LIVESTOCK<sup>1</sup>

J. B. O'NEIL<sup>2</sup> AND J. M. BELL<sup>3</sup>

*University of Saskatchewan, Saskatoon, Sask.*

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## ABSTRACT

A commercial product bearing the trade name "Feed-Ani" was fed at the rate of 2 per cent to growing chicks and growing mice that were fed commercial chick starter mash and growing-swine rations, respectively. The growth of the chicks and their relative growth efficiencies were depressed by an amount approximating the dilution with "Feed-Ani". In the mouse-feeding trials, "Feed-Ani" increased feed consumption by an average of 13 per cent on all diets tested, but in no instance did it improve gains in weight. The use of "Feed-Ani" led to a significant decrease in feed efficiency.

During the past two years a feed supplement bearing the trade name "Feed-Ani" has been distributed and sold in Canada. Rather spectacular claims have been made regarding its effect on animal and poultry health. It has been said, or inferred, favourably to influence cannibalism, hatchability, liveweight gains, butterfat percentage, calf scours and certain other production problems. These observations appear not to have been based upon well-controlled experiments.

"Feed-Ani" is stated to be a naturally-occurring mineral deposit obtained in Nevada, U.S.A. It contains about 80 per cent silica compounds and small or trace quantities of the "essential" minerals. Attention has been drawn to an 'organic component', the value of which has not yet been elucidated. Fluorine is present at a level of about 0.3 per cent. Current recommendations state that "Feed-Ani" should be added to farm rations at the rate of 2-5 per cent in addition to the regular supplements.

The present report contains the results of feeding trials with growing chicks and growing mice that were fed commercial chick starters and growing-swine rations respectively, and to which 'recommended' levels of "Feed-Ani" were added.

## MATERIALS AND METHODS

### *Chick Experiment*

Eight groups of 21 or 22 crossbred chicks (New Hampshire × Barred Plymouth Rock) were sight-sexed, randomized at one day of age and placed in separate compartments of an electric starter battery equipped with raised wire-mesh floors. Each group was represented by one sex only. The control lots were comprised of one group of each sex for each of the two commercial starter mash studies. "Feed-Ani" was mixed into each of the starter mashes at the rate of 2 per cent and fed to the remaining four groups of chicks. Both of the commercial feeds contained antibiotics at currently recommended levels.

<sup>1</sup> Joint contribution from the Departments of Poultry Husbandry and Animal Husbandry.

<sup>2</sup> Associate Professor of Poultry Husbandry.

<sup>3</sup> Professor of Animal Husbandry.

Individual weights on the chicks were taken initially and weekly thereafter until the test was terminated at the end of the seventh week. Feed and water were supplied ad libitum. Feed was weighed back each week after the chicks were weighed. Mortality was recorded.

The floor space per chick was as follows: 0-3 weeks, 0.2 square feet; 3-7 weeks, 0.4 square feet. When five weeks of age the chicks were transferred to a broiler battery for the remaining two weeks.

### *Mouse Experiment*

An experiment for the evaluation of yeast-whey provided an opportunity to test "Feed-Ani" under a variety of conditions. All rations contained 18 per cent protein but they varied with respect to source of supplemental protein, vitamin B-complex adequacy and types of basal ingredients. All diets were supplemented with vitamins A and D and with a complex mineral mixture (1). No antibiotics were included.

The experiment was of a  $2 \times 3 \times 4$  factorial design in five replicates. The basal portions were either grain ( $\frac{2}{3}$  wheat,  $\frac{1}{3}$  oats) or semi-purified ingredients (70 starch, 3 sugar, 7 cellulose, 3 crisco, 13 vitamin-free casein). Each diet employed one of four protein supplements: (a) yeast-whey, (b) yeast-whey plus methionine, (c) skim milk powder and (d) soybean oil meal plus fish meal (50 : 50). All rations in the fifth replicate were admixed with 2 per cent "Feed-Ani".

Growth and feed records were obtained on weanling male mice during the customary 2-week assay period. The animals were housed in individual cages, with wire-mesh floors. Feed and water were provided ad libitum. The animals were randomized to cages and to diets, within replicates.

## EXPERIMENTAL RESULTS AND DISCUSSION

### *Chick Experiment*

The growth patterns of the chicks on the two commercial feeds, whether supplemented with "Feed-Ani" or not, were very similar although, as might be expected, the males consistently outweighed the females. Since there was no disparity in sex numbers, the growth data were combined according to treatments and are shown in Table 1 along with the percentage difference in response.

TABLE 1.—EFFECT OF "FEED-ANI" ON CUMULATIVE GAINS IN CHICKS FED COMMERCIAL STARTER RATIONS (GAINS IN GRAMS)

Treatment	Age in weeks							
	0	1	2	3	4	5	6	7
Commercial starters	39.9	63.1	108.4	182.2	283.6	375.8	511.1	648.7
Commercial starters + 2 per cent "Feed-Ani"	39.4	60.9	102.4	176.8	271.6	364.2	494.0	634.1
Difference, per cent	—	3.5	5.5	3.3	4.2	3.1	3.4	2.2



It will be seen that the growth of the chicks on the supplemented starters was uniformly less throughout the trial, ranging from 2.2 to 5.5 per cent below the gains made by the unsupplemented chicks. This trend, while small, was very consistent, thus indicating that the growth of these chicks was depressed in approximate proportion to their supplementation by "Feed-Ani".

Mortality was very low, being two and three male chicks for the unsupplemented and supplemented groups, respectively. Of this total, three were removed at the end of the fifth week because of severe perotic conditions, one of the three being from the unsupplemented groups. No feather picking or cannibalism was observed.

While the growth of chicks on the commercial starters alone was slightly greater, it is of interest to compare the relative growth efficiencies of the two groups. Table 2 lists these figures as units of feed per unit of gain in body weight on a cumulative basis. In addition, the differences in percentages between treatments are shown.

TABLE 2.—EFFECT OF "FEED-ANI" ON CUMULATIVE FEED EFFICIENCIES IN CHICKS FED COMMERCIAL STARTER RATIONS (GRAMS FEED/GRAM GAIN)

Treatment	Feeding period in weeks							Av. gain per chick (0-7 weeks)	Av. feed per chick (0-7 weeks)
	0-1	0-2	0-3	0-4	0-5	0-6	0-7		
								gm.	gm.
Commercial starters	2.38	2.30	2.38	2.54	2.87	2.84	2.93	609	1784
Commercial starters + 2 per cent "Feed-Ani"	2.55	2.40	2.41	2.64	2.92	2.92	2.99	595	1778
Difference, per cent	6.9	4.3	1.7	3.9	1.7	2.8	2.0	2.3	0.3

The efficiency data show that chicks receiving only commercial starters required less feed per unit gain in body weight. The differences are in the same direction as the growth data shown in Table 1, though they tend to decrease with time. It is improbable that the differences in feed efficiency were due to palatability, since there was only 0.3 per cent difference in feed consumption per chick for the period. The decrease in gains (per unit feed) again appears to approximate the dilution of the rations by "Feed-Ani".

Because of slower growth and lower efficiency of feed conversion, the cost per pound of gain was increased by the incorporation of "Feed-Ani" by 4.4 per cent over the 7-week period.

### *Mouse Experiment*

During the mouse feeding trials there were no apparent differences in the outward appearance or behaviour of the mice, but analyses of the resulting data revealed important effects of "Feed-Ani" on gains and feed efficiency. "Feed-Ani" increased feed consumption by an average of 13 per cent for all diets tested, but in no instance did it give improved gains (Table 3). This relationship indicates that there was a significant decrease

TABLE 3.—EFFECT OF "FEED-ANI" ON GROWTH AND FEED CONSUMPTION OF MICE FED RATIONS DIFFERING IN BASAL COMPONENTS, VITAMIN SUPPLEMENTS OR PROTEIN SUPPLEMENTS (WEIGHTS IN GRAMS)

Treatments	Observed gain		Feed consumption		Gains adjusted for feed	
	Control	"Feed-Ani"	Control	"Feed-Ani"	Control	"Feed-Ani"
All rations	10.6 (1.0)*	10.8	41.9 (2.4)	47.3	10.9 (0.8)	9.5
Grain basal	11.9 (1.4)	11.9	45.4 (3.4)	52.3	11.2 (1.1)	9.2
Semi-purified basal	9.3	9.8	38.3	42.3	10.6	10.0
No vitamin supplement	10.2 (1.8)	9.7	41.1 (4.1)	46.6	10.8 (1.3)	8.6
Brewers' yeast, 2 per cent	11.3	11.6	43.3	48.3	11.2	10.0
Yeast-whey, 2 per cent	10.2	11.1	41.2	47.0	10.7	9.9
Yeast-whey	10.0 (2.0)	10.9	41.4 (4.8)	47.7	10.5 (1.5)	9.5
Yeast-whey + methionine	10.8	11.1	40.7	46.3	11.5	10.1
Skim-milk powder	11.3	11.6	42.8	47.8	11.3	10.2
Soybean oilmeal + fishmeal	10.2	9.7	42.6	47.5	10.3	8.4

\* Figures in parentheses are the necessary differences at  $P=0.05$  per cent.

in feed efficiency. This effect is illustrated in the last two columns of Table 3, which show gains after statistical correction for differences in feed intakes.

When the results are considered separately according to the type of basal ration employed, it is seen that greater depression in efficiency due to "Feed-Ani" occurred when natural grains were used. Although the same trend exists on the semi-purified diets, it may be that lack of 'real' differences is attributable to the generally lower feed intakes on these diets, resulting in smaller margins of nutrients being available for growth purposes over and above the maintenance needs.

The relationship of "Feed-Ani" to the inclusion of vitamin supplements in the rations is of interest in view of certain of the merits claimed. When no vitamin (B-complex) supplement was added, "Feed-Ani" caused a pronounced reduction in adjusted gain (or in feed efficiency), and while the depressive effects of "Feed-Ani" were not significant in the presence of brewers' yeast or yeast-whey, there was obviously no advantage in including the product.

Some differences were evident in the effects of "Feed-Ani" when used in conjunction with different protein supplements. The responses were all unfavourably affected by "Feed-Ani", but the unfavourable effects were more serious when soybean oilmeal-fishmeal supplements were used instead of the milk by-products.

From the standpoint of economics, the increased feed cost due to inclusion of 2 per cent "Feed-Ani", together with the decreased feed efficiency, resulted in an over-all increase in cost per unit gain of 15-20 per cent over the cost of an ordinary commercial ration. There is thus little indication that "Feed-Ani" is of value in the rations of growing chicks or

non-ruminant farm animals when it is included at recommended levels in otherwise adequate rations based largely on Western Canadian grains. Further studies with poultry are in progress.

#### ACKNOWLEDGMENT

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# THE EFFECT OF $P^{32}$ RADIATION ON CROP GROWTH AND PHOSPHORUS UPTAKE<sup>1</sup>

## I. GREENHOUSE STUDIES

E. PENNER<sup>2</sup>

*Department of Soil Science, University of Saskatchewan, Saskatoon, Sask.*

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### ABSTRACT

Thatcher wheat was grown for 20-, 30- and 40-day periods under greenhouse conditions. Radioactive  $NH_4H_2PO_4$  (24 lb.  $P_2O_5$  per acre) was applied in solution and in granular form at levels of  $P^{32}$  ranging from 0-3600  $\mu C$   $P^{32}$  per gram  $P^{31}$ . When applied in solution, radiation effects were shown to occur at levels as low as 12  $\mu C$   $P^{32}$  per gram  $P^{31}$  causing alterations in plant weight and soil phosphorus absorption. No deviations occurred when radioactive  $NH_4H_2PO_4$  was applied in granular form. A further experiment included several common cereal crops. Radioactive  $NH_4H_2PO_4$  was applied in granular form at levels ranging from 0-4000  $\mu C$   $P^{32}$  per gram  $P^{31}$ . Some reduction in soil phosphorus absorption occurred with oats and barley at 8 and 80  $\mu C$   $P^{32}$  per gram  $P^{31}$ , respectively. Wheat showed no variations in any of the criteria examined. No alterations in soil phosphorus/fertilizer phosphorus ratios occurred in any of the experiments. Under greenhouse conditions, when the fertilizer is applied in granular form, changes in growth, or uptake of phosphorus in cereal crops due to radioactivity, do not appear to seriously affect the value of the tracer method.

It is of considerable interest to the research worker using radioactive tracer techniques in biological systems whether the emitted radiations seriously interfere with the growth processes of living tissue. Although the complete avoidance of radiation effects is improbable, it has been tacitly assumed that the deviations attributable to the radioactive nature of the tracer are sufficiently small at low specific activities so as not to invalidate its use. It appears from recent reports with regard to  $P^{32}$  in phosphate fertilizer research that in some cases this assumption is violated, and as a result the validity of data obtained from phosphate tracer experiments may be questioned. The experimental results obtained by various workers investigating this possibility are not consistent, indicating that the conditions of the experiment may be an important factor influencing the sensitivity of the plant tissue to ionizing radiations.

Russel and Martin (12) have shown that in nutrient solution considerable interference may occur to the growth of young barley plants at activity levels as low as 10  $\mu C$   $P^{32}$  per litre. Reductions in the total phosphorus content of roots were as large as 40 per cent at the 50  $\mu C$   $P^{32}$  per litre level as compared to the 0.5  $\mu C$  level. Russel *et al.* (13) have shown in soil studies that plant weight, used by Dion *et al.* (5) and Hendricks and Dean (6), may not be an adequate criterion for the assessment of radiation effects. Under the conditions of the experiment reported by Russel, soil phosphorus/fertilizer phosphorus ratios may be altered in the absence of variations in the dry weight of plant material.

In soil studies with barley as the test plant, Bould *et al.* (3) report increases in the dry weight of shoots after 22 days of growth for applications

<sup>1</sup> Contribution from the Department of Soil Science, University of Saskatchewan. The material contained herein is part of a thesis submitted to the College of Graduate Studies in partial fulfilment for the degree of MSc.

<sup>2</sup> Graduate Student.

of  $P^{32}$  as low as  $5\mu\text{C } P^{32}$  per pot, while soil phosphorus/fertilizer phosphorus ratios were affected only at the highest level— $400\mu\text{C } P^{32}$  per pot. Further studies in the same soil, with the same crop, failed to show any effect on shoot growth, but for this experiment growth was less vigorous.

Radiation effects on the uptake of fertilizer have been reported by Blume (2) at a specific activity as low as  $20\mu\text{C } P^{32}$  per gram  $P^{31}$  with barley for 54 days' growth and with oats at  $50\mu\text{C } P^{32}$  per gram  $P^{31}$  for 42 days. The dry weights of shoots were only altered at much higher levels of activity.

Both Blume (2) and Bould *et al.* (3) stressed the smallness of the deviations even though sometimes statistically significant.

### EXPERIMENTAL METHODS

In principle the method used to investigate radiation effects is the same as has been used by other workers. The treatments consisted of constant applications of  $P^{31}$  at various  $P^{32}$  levels. These levels were selected to approximate studies at other institutions as well as to include those normally used in phosphate fertilizer tracer research.

The radioactive phosphorus was obtained from the Isotope Branch of the National Research Council as  $\text{H}_3\text{PO}_4$  prepared from sulphur by neutron bombardment, and was incorporated into  $\text{NH}_4\text{H}_2\text{PO}_4$  by the

TABLE 1.—EXPERIMENT A—THE EFFECT OF  $P^{32}$  ON THATCHER WHEAT GROWN IN THE GREENHOUSE WHEN THE RADIOACTIVE FERTILIZER IS APPLIED IN SOLUTION

Microcuries $P^{32}$ /gm. $P^{31}$	Harvests*	0	12	120	1200	L.S.D.
Shoot Wt. gm./pot	1.	0.46	0.45	0.46	0.40	0.04
	2.	1.29	1.42	1.41	1.32	N.S.
	3.	2.71	2.17	2.48	2.61	0.33
Total P mg./pot	1.	2.75	2.65	2.60	2.26	N.S.
	2.	5.22	6.26	5.76	5.86	N.S.
	3.	11.3	9.2	11.1	11.5	N.S.
Soil P mg./pot	1.	—	1.79	1.82	1.55	N.S.
	2.	—	4.18	3.75	3.86	N.S.
	3.	—	6.27	7.83	8.09	1.3
Fertilizer P mg./pot	1.	—	0.77	0.78	0.70	N.S.
	2.	—	2.09	2.02	1.99	N.S.
	3.	—	3.09	3.31	3.43	N.S.
Per cent of Fertilizer P absorbed	1.	—	2.64	2.66	2.41	—
	2.	—	7.14	6.91	6.82	—
	3.	—	10.6	11.4	11.8	—
Ratio Soil P Fert. P	1.	—	3.01	2.49	2.56	N.S.
	2.	—	2.03	1.90	1.94	N.S.
	3.	—	2.11	2.36	2.39	N.S.

\* 1. 20-day harvest.  
2. 30-day harvest.  
3. 40-day harvest.

method used by Mitchell *et al.* (10). All  $P^{32}$  dilutions were based on the routine analytical report accompanying the isotope shipment and no attempt was made to check the absolute activity; however, there was good relative agreement between the various shipments received.

In Experiments A and B, Thatcher wheat was grown in 8 pounds of Melfort Silty Clay Loam<sup>1</sup> contained in glazed one-gallon crocks. The planting and growing procedure was similar for both experiments with the exception of the method of fertilizer application. In Experiment A, the radioactive fertilizer was added in solution at seed level one inch below the soil surface; in Experiment B, the fertilizer was applied in granular form adjacent to the seeds also one inch below the soil surface. The fertilizer application was 24 lb.  $P_2O_5$  per acre calculated on an area basis. In Experiment A, the treatments consisted of differential levels of  $P^{32}$ —0, 12, 120 and 1200  $\mu$ C  $P^{32}$  per gram  $P^{31}$ ; two additional levels of  $P^{32}$  were included in Experiment B—600 and 3600  $\mu$ C  $P^{32}$  per gram  $P^{31}$ .

Ten seeds were planted in each pot but only five plants were allowed to grow after emergence. This was done in order to ensure five vigorous plants per pot. Each treatment was replicated five times and the above-ground plant material from each pot constituted one sampling unit. The plants were grown during the winter in a greenhouse maintained at  $70^\circ F. \pm 8^\circ$ . Natural illumination was supplemented to simulate the length of

<sup>1</sup> A detailed description of the soils used may be found in Soil Survey Report No. 12, Department of Soil Science, University of Saskatchewan.

TABLE 2.—EXPERIMENT B—THE EFFECT OF  $P^{32}$  ON THATCHER WHEAT GROWN IN THE GREENHOUSE WHEN THE RADIOACTIVE FERTILIZER IS APPLIED IN THE GRANULAR FORM

Microcuries $P^{32}$ /gm. $P^{31}$	Harvests*	0	12	120	600	1200	3600	L.S.D.
Shoot Wt. gm./pot	1.	1.11	1.13	1.22	1.21	1.16	1.20	N.S.
	2.	2.42	2.53	2.39	2.49	2.29	2.37	N.S.
Total P mg./pot	1.	4.94	5.46	6.04	5.68	5.72	5.84	N.S.
	2.	10.7	11.6	10.5	11.5	10.8	11.6	N.S.
Soil P mg./pot	1.	—	2.63	3.21	2.75	2.94	3.01	N.S.
	2.	—	6.36	6.06	6.45	6.39	6.40	N.S.
Fertilizer P mg./pot	1.	—	2.82	2.83	2.93	2.78	2.82	N.S.
	2.	—	5.24	4.38	5.07	4.37	5.24	N.S.
Per cent of Fert. P absorbed	1.	—	9.66	9.69	10.05	9.52	9.67	—
	2.	—	17.9	15.0	17.4	15.0	17.9	—
Ratio Soil P Fert. P	1.	—	0.96	1.14	0.96	1.07	1.07	N.S.
	2.	—	1.23	1.39	1.27	1.48	1.22	N.S.

\* 1. 30-day harvest.

2. 40-day harvest.



day in a normal growing season. Harvests were taken after 20, 30 and 40 days of growth in Experiment A, and after 30 and 40 days in Experiment B. Plant weights were established on the basis of oven dry (110° C.) weights.

The treatments in Experiment C consisted of differential  $P^{32}$  levels, 0, 8, 80, 800 2400 and 4000  $\mu\text{C}$   $P^{32}$  per gram  $P^{31}$  for four common cereal crops, namely: Thatcher wheat, Montcalm barley, Fortune oats and Prolific rye. The soil used was Asquith Fine Sandy Loam but in every other respect the planting and growing procedure was similar to Experiment B. In this experiment the harvest was taken after 40 days of growth.

Quantitative estimations of  $P^{32}$  were made according to the method outlined by MacKenzie and Dean (9) with the exception of the preparation of standards which were prepared according to Kristjanson *et al.* (8). Where less than three grams of oven dry plant material was available the samples were wet ashed and counted in solution (14). All  $P^{32}$  samples were counted for a sufficient period to reduce the probable error due to counting to less than 1 per cent.

The oven dried plant material was wet ashed according to the method of Brenner and Harris (4) and the total phosphorus was determined colorimetrically as outlined by Kitson and Mellon (7). The soil phosphorus was calculated from the total and fertilizer phosphorus analyses with no allowance for the small amount of seed phosphorus contained in each plant.

TABLE 3.—THE EFFECT OF  $P^{32}$  ON LEAF LENGTHS\*

Microcuries $P^{32}$ /gm. $P^{31}$	Harvests**	0	12	120	600	1200	3600	L.S.D.
First leaf	1.	19	18	18	18	18	18	N.S.
	2.	—	—	—	—	—	—	—
	3.	18	17	17	—	17	—	N.S.
Second leaf	1.	34	35	37	34	35	34	N.S.
	2.	38	39	39	39	38	39	N.S.
	3.	36	34	36	—	35	—	N.S.
Third leaf	1.	38	37	39	37	37	37	N.S.
	2.	44	42	42	44	44	45	N.S.
	3.	40	40	40	—	39	—	N.S.
Fourth leaf	1.	40	40	42	41	41	41	N.S.
	2.	50	51	53	51	50	53	N.S.
	3.	48	48	49	—	49	—	N.S.
Fifth leaf	1.	40	39	41	43	40	43	N.S.
	2.	61	59	62	63	61	64	N.S.
	3.	62	60	59	—	63	—	N.S.

\* The leaf length measurements are the distance from the tip of the leaf to the base of the plant in cm.

\*\* 1. 30-day harvest - Experiment B.  
2. 40-day harvest - Experiment B.  
3. 40-day harvest - Experiment A.

## EXPERIMENTAL RESULTS

The results of Experiments A, B and C are given in Tables 1, 2 and 4, respectively. Table 3 gives the results of the leaf length measurements for the 40-day harvest in Experiment A and for the 30- and 40-day harvests in Experiment B.

In Experiment A at the 20-day harvest the weight of shoots was significantly decreased at the highest  $P^{32}$  level,  $1200\mu\text{C } P^{32}$  per gram  $P^{31}$ , but not for the 30-day harvest. At 40 days, a 20 per cent decrease in shoot weight occurred at the  $12\mu\text{C}$  level compared to the check treatment, while higher activity levels also showed a downward trend but the differences were not statistically significant. The  $12\mu\text{C}$  treatment at the 40-day harvest was also lower in total phosphorus, fertilizer phosphorus and soil phosphorus content than higher levels. The soil phosphorus/fertilizer phosphorus ratio was also lower but of all these criteria only the decrease in soil phosphorus was significant.

TABLE 4.—EXPERIMENT C\*—THE EFFECT OF  $P^{32}$  ON CEREAL CROPS UNDER GREENHOUSE CONDITIONS FOR THE GRANULAR METHOD OF FERTILIZER APPLICATION

Microcuries $P^{32}$ /gm. $P^{31}$	0	8	80	800	2400	4000	L.S.D.
Shoot Wt. gm./pot							
Wheat	5.20	5.36	5.17	5.32	4.99	5.06	N.S.
Barley	6.36	6.85	6.48	6.74	6.67	6.45	N.S.
Oats	4.99	4.63	4.99	4.66	5.01	4.86	N.S.
Rye	4.93	5.16	5.09	5.04	5.15	6.01	N.S.
Total P mg./pot							
Wheat	16.29	16.57	17.09	16.66	16.17	15.86	N.S.
Barley	19.14	19.58	19.04	20.22	19.93	18.70	N.S.
Oats	17.28	14.91	16.95	15.68	16.48	16.33	N.S.
Rye	18.38	17.89	18.69	18.60	18.73	21.15	N.S.
Soil P mg./pot							
Wheat	—	9.39	9.40	9.02	8.78	8.40	N.S.
Barley	—	10.85	9.97	11.24	11.02	10.14	0.52
Oats	—	8.76	10.75	9.73	10.07	10.02	1.23
Rye	—	10.03	10.74	11.00	11.08	12.10	N.S.
Fertilizer P mg./pot							
Wheat	—	7.18	7.69	7.63	7.40	7.46	N.S.
Barley	—	8.73	9.07	8.98	8.91	8.56	N.S.
Oats	—	6.16	6.21	5.95	6.40	6.31	N.S.
Rye	—	7.86	7.94	7.60	7.65	9.05	1.00
Per cent of Fertilizer absorbed							
Wheat	—	24.59	26.34	26.12	25.31	25.53	—
Barley	—	29.87	31.03	30.74	30.49	29.32	—
Oats	—	21.09	21.25	20.31	21.92	21.60	—
Rye	—	26.91	27.20	26.01	26.22	30.99	—
Ratio Soil P Fert. P							
Wheat	—	1.31	1.22	1.18	1.19	1.13	N.S.
Barley	—	1.25	1.11	1.25	1.24	1.19	N.S.
Oats	—	1.43	1.74	1.65	1.58	1.60	N.S.
Rye	—	1.28	1.36	1.49	1.45	1.34	N.S.

\* Harvested after 49 days of growth.

Experiment B, which was conducted similarly to Experiment A with the exception of the method of fertilizer application, showed no variation in any of the criteria examined. The results are given in Table 2. Except for the addition of a 600 and 3600 $\mu$ c treatment the levels of activity used were the same as used in Experiment A.

Blume *et al.* (1) have shown the usefulness of leaf measurements as a sensitive criterion for radiation injury. Table 3 gives the leaf measurements for Experiment A at 40 days and Experiment B at 30 and 40 days. The leaf measurements at all levels of activity showed no evidence of radiation injury. In soil studies of this type it appears other criteria are considerably more sensitive to alteration due to the presence of radioactive phosphorus.

The results of Experiment C are given in Table 4. This experiment was designed to compare the sensitivity of wheat, barley, oats and rye over a range of tracer levels. Although no deviations occurred in plant weight, total phosphorus uptake, or in soil phosphorus/fertilizer phosphorus ratios, there is further evidence that there may sometimes be interference with soil phosphorus absorption. In the oat experiment, at the 8 $\mu$ c level of activity, soil phosphorus was significantly lower than at all higher activities with the exception of the 800 $\mu$ c treatment. In the barley experiment a significant decrease in soil phosphorus uptake occurred at the 80 $\mu$ c level as compared to both lower and higher levels of P<sup>32</sup>. Fertilizer phosphorus uptake was significantly increased at the highest level in the case of rye, but this was of less importance since 4000 $\mu$ c P<sup>32</sup> per gram P<sup>31</sup> was a considerably higher activity than need normally be used.

## DISCUSSION AND CONCLUSIONS

The deviations at the 40-day harvest in Experiment A cannot be easily discounted, first, because of the occurrence at low tracer levels (12 $\mu$ c P<sup>32</sup> per gram P<sup>31</sup>) and second, because of the magnitude of effects. A comparison between Experiment A and Experiment B, both for 40 days, as well as Experiment C for wheat, would indicate that these effects are related to the method of fertilizer application. There appears to be no ready explanation for the greater effect of radioactive phosphorus when applied in solution as compared to application in the granular form. Where significant deviations in plant growth or in uptake of phosphorus were observed they occurred at the lower levels of activity.

Soil phosphorus/fertilizer phosphorus ratios were not affected, indicating that in those instances where decreases were noted in soil phosphorus, the fertilizer phosphorus also decreased even though statistical treatment failed to show it significant. This has important implications since this ratio seems to have a bearing on the problem of soil phosphorus availability (11).

Under greenhouse conditions cereal crops may be expected to be very similar in their sensitivity to radioactive phosphorus, at least when the fertilizer is applied in the granular form (Table 4). Out of the many criteria examined it is only in the uptake of soil phosphorus that deviations of any significance were observed.



In general, the data suggest that the conditions under which the experiment is carried out is as important as the levels of activity used in affecting plant growth or uptake of phosphorus. While marked deviations occurred in the data for the 40-day harvest where the fertilizer was applied in solution, relatively minor effects occurred where the fertilizer was applied in the granular form. The possibility of some radiation effects should not be overlooked. However, such effects do not appear to be sufficiently serious to invalidate the use of radioactive phosphorus as a tracer in fertilizer studies.

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# THE USE OF CHEMICALS AND OF MALE STERILITY TO CONTROL POLLEN PRODUCTION IN CORN<sup>1</sup>

F. S. WARREN<sup>3</sup> AND F. DIMMOCK<sup>2</sup>

*Central Experimental Farm, Ottawa, Canada*

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## ABSTRACT

Two methods of controlling pollen production in corn—spraying with M.H. and introducing cytoplasmic male-sterility—are under investigation by the Division of Forage Plants, Central Experimental Farm, Ottawa.

Three hybrids were sprayed with solutions of M.H. in concentrations of 0.025, 0.05, 0.1 and 0.15 per cent over a two-year period. Pollen production was not inhibited by any treatment, nor was there any other noticeable plant response.

Cytoplasmic male-sterility was transferred to two early inbred lines by crossing these to a series of male-sterile single crosses and backcrossing to the inbred parent. Normal plants were obtained by selecting for low sterility. Seasonal conditions influenced pollen production of the crosses.

## INTRODUCTION

The present method of detasseling the female parent in producing hybrid corn seed is tedious with costs amounting to \$10.00 to \$20.00 an acre and involving expenditures estimated at \$200,000 annually in Canada. Methods which might eliminate hand detasseling are being studied; two of these—spraying with maleic hydrazide (M.H.) and introducing male sterility—are under investigation at the Division of Forage Plants, Central Experimental Farm, Ottawa.

## LITERATURE REVIEW

### *Maleic Hydrazide (M.H.)*

A great variety of materials have been used to inhibit pollen production in corn, but of these only M.H. has been reported to prevent pollen formation without severely damaging the plants.

Naylor (10) eliminated anther development in greenhouse hybrids sprayed with 0.025 per cent M.H. when 35 days old. Field plantings sprayed when the tassels were 5 inches long were also male sterile. Naylor and Davis (11) reported pollen sterility without affecting vegetative growth following applications of 0.025 per cent M.H. during microsporogenesis.

Josephson (8), using M.H. in concentrations of 0.2, 0.1 and 0.05 per cent, and Denisen and Haber (1) with 0.1, 0.05 and 0.025 per cent, were in agreement that pollen sterility could be effected only at the expense of extreme reduction in yield.

Eskew and Willard (2) treated hybrid corn when 7 to 12 inches high with 2 to 8 pounds per acre of M.H. in 10 gallons of water and observed that some pollen was produced, even when growth was severely affected.

Moore (9) in trials with sweet corn found that a solution of 600 p.p.m. caused tassel sterility accompanied by stubby ears and reduced yield.

<sup>1</sup> Contribution of the Division of Forage Plants, Central Experimental Farm, Ottawa.

<sup>2</sup> Agricultural Research Officer, in charge of corn breeding and investigations.

<sup>3</sup> Agricultural Research Officer, Assistant in corn breeding and investigations.

### *Cytoplasmic Male-sterility*

Rhoades (12) in 1933 described a form of pollen sterility found in an ear of Peruvian corn collected by R. A. Emerson and F. D. Richey. Due to the breeding behaviour of the material, he concluded that the sterility was dependent on a cytoplasmic condition maternally inherited and independent of genic influence.

Josephson and Jenkins (7) found considerable variation in the degree of sterility produced by the action of the cytoplasmic contribution. The pollen parent was able to exert some influence on the sterility and, apparently, at least two genes were responsible. In addition, environmental conditions affected the degree of sterility.

Jones (4), Jones and Everett (5), and Jones and Mangelsdorf (6), have reported extensively on the breeding behaviour of several cytoplasmic male-sterile cultures. They noted that cytoplasmic male sterility could be transferred with varying success to inbred strains without altering other characteristics. Segregation for sterility in these crosses and backcrosses was present to the extent that sub-lines could frequently be established either high or low in sterility. Some inbreds had the ability to overcome the pollen sterile influence.

Jones and Mangelsdorf (6) suggested a method of utilizing the cytoplasmic factor in hybrid seed production. The male sterility is transferred to a selected inbred which is used as the female parent of a single cross. The resulting male-sterile single cross becomes the female parent of the double cross. The commercial hybrid seed is then made up of a mixture of male-sterile double cross and about 25 per cent of normal seed of identical genotype and quality. Mixing the seed could be eliminated by incorporating a satisfactory pollen-restoring gene in the male parent single cross.

Rogers and Edwardson (13) reported slightly higher yields on the average from a number of male-sterile single and double crosses in comparison with similar normal hybrids.

## EXPERIMENTAL MATERIAL AND METHODS

### *Maleic Hydrazide*

In 1951 a plot of the hybrid Canbred 210, was sprayed with M.H. in concentrations of 0.025, 0.05, and 0.1 per cent. Triton X 100 was used as a wetting agent and the plants were sprayed to run-off with a hand sprayer when they were about thirty inches high. Twenty-five plants were sprayed at each rate in a randomized triple replication. At appropriate times comparisons were made with untreated check plots for pollen productivity, foliage injury and yield of seed.

The following year hybrids Canbred 230 and Canbred 260 were sprayed with M.H. in concentrations of 0.025, 0.05, 0.1, and 0.15 per cent. Forty-five plants of Canbred 230 were treated for each rate in two replications on July 17. The Canbred 260 plot was planted to provide a graded series of growth stages from 12 to 35 inches in height at the time of spraying. Thirty-six plants representing all growth stages were included in each treatment.



Observations were made on all plots for pollen production, foliage injury and yield. When plants in the control plots were shedding pollen, tassel bags were placed on a number of plants in each treated plot for pollen examination.

### *Cytoplasmic Male-sterility*

Seed of five male-sterile single crosses was obtained from D. F. Jones, New Haven, Conn., in 1950. These single crosses proved to be completely male sterile and crosses were made on all of them, using inbreds 103 and 106 as the pollen parents. Inbred 103 normally produces limited amounts of pollen while 106 regularly has an abundance of pollen. Extreme differences in tassel type are typical, as illustrated in Figure 1.

The following year selected plants of the single cross  $\times$  inbred populations were crossed a second time with inbreds 103 and 106 respectively. Tassel samples were taken from these selected plants and examined for degree of pollen abortion.

In 1952, three ears of each single cross  $\times$  inbred<sup>2</sup> population from highly pollen-sterile plants were grown ear-to-row, and again crossed by the 103 or 106 parents. Similarly three ears from plants low in sterility were grown ear-to-row and pollen-fertile plants of this group were self-pollinated. Glassine tassel bags were placed on all plants grown from both groups of ears and examined each day for pollen production.

## EXPERIMENTAL RESULTS

### *Maleic Hydrazide*

Treated rows in the 1951 experiment were indistinguishable from the control rows. Pollen was produced abundantly by all plants. There was no visible injury to the tassel or foliage. Heights and yields did not differ statistically.

TABLE 1.—CLASSIFICATION OF SINGLE CROSS  $\times$  INBRED PLANTS ACCORDING TO DEGREE OF POLLEN STERILITY

Cross	Numbers of plants in classes of per cent pollen sterility							Total number of plants
	0-15	16-30	31-45	46-60	61-75	76-90	91-100	
(A158 $\times$ WF9) 103	—	1	—	—	—	2	8	11
(A158 $\times$ WF9) 106	—	—	—	—	4	1	10	15
(A $\times$ A158) 103	—	—	—	2	—	1	7	10
(A $\times$ A158) 106	—	—	—	—	1	2	10	13
(M14 $\times$ WF9) 103	—	1	1	1	3	4	7	17
(M14 $\times$ WF9) 106	—	—	—	—	—	3	11	14
(C106 $\times$ WF9) 103	—	—	—	—	—	—	2	2
(C106 $\times$ WF9) 106	3	2	—	1	—	—	4	10
Total	3	4	1	4	8	13	59	92



FIGURE 1. Tassel types of inbreds. *Left*—Inbred 103, poor pollen producer. *Right*—Inbred 106, good pollen producer.

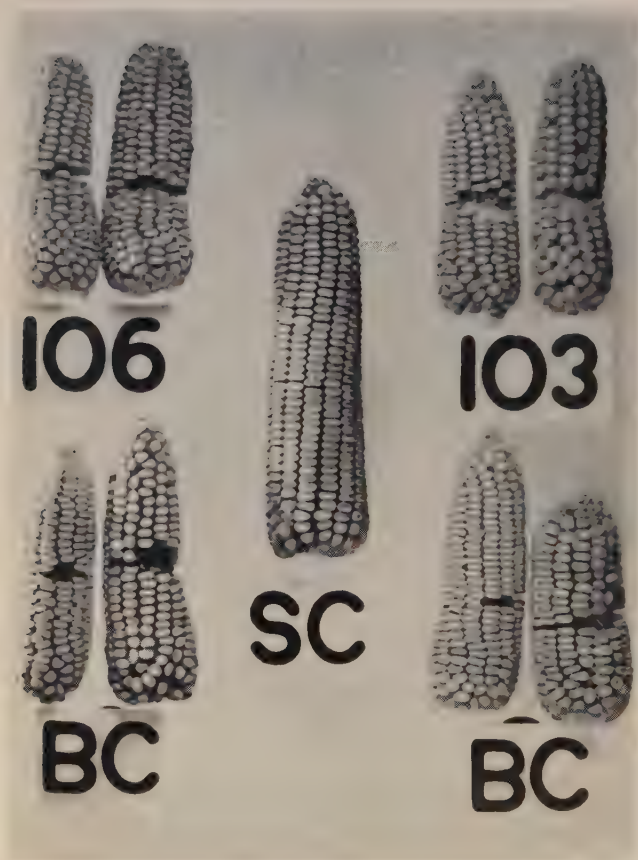


FIGURE 2. Typical ears of inbreds and hybrids. *Centre*—single-cross parent. *Left*—inbred 106 and single cross  $\times 106^2$  (B.C.). *Right*—inbred 103 and single cross  $\times 103^2$  (B.C.).



The 1952 results were similar to those of 1951. Pollen was produced in quantity by even those plants which were in the earliest stage of development when sprayed. There were no noticeable effects of the treatment at any concentration of M.H.

### *Cytoplasmic Male-sterility*

Plants of the various single cross  $\times$  inbred crosses grown in 1951 were mostly highly sterile. The degree of sterility estimated from iodine smear examination of the pollen of a number of plants is shown in Table 1.

The plants grown from the three ears of each cross selected as highly sterile produced no pollen in 1952. Approximately 650 plants were grown from the strains selected for low sterility and of these 20 shed some pollen, but only nine produced seed from self-pollination. Six of these ears were from crosses by inbred 103 and the remaining three had inbred 106 in the combination.

Phenotypically the single cross  $\times$  inbred<sup>2</sup> plants resembled the respective inbred parents very closely. Figure 2 shows the ear type of an original single-cross parent, along with ears of parent inbreds 103 and 106, and their hybrids.

## DISCUSSION

### *Maleic Hydrazide*

In these experiments M.H. treatment produced no pollen inhibition and no apparent effect of any kind on the plants. The concentrations of M.H. were similar to those which gave marked response when used by Naylor (10) and Eskew and Willard (2). Earlier application might have been effective, although in the case of Canbred 260 the treatment included a wide range of growth stages without producing a response from any plants.

Josephson (8) noted a varietal response, indicating that the three hybrids used in the present experiment could be less sensitive than the varieties reported on by other investigators. It is possible that the dosage may have to be increased to be effective in this area.

### *Cytoplasmic Male-sterility*

The breeding program seems to be proceeding satisfactorily toward the establishment of two early-maturing, male-sterile inbreds. Although considerable pollen was produced by all lines in 1951 probably as the result of seasonal influences, highly sterile plants were obtained in all crosses. These produced no pollen the following year.

Usually the better pollen producing inbreds are more difficult to convert to the male sterile condition. Inbred 106, an abundant pollen producer, seems to be an exception. The data in Table 1 show that 67 per cent of the plants having the 106 parentage were in the highly sterile classification (91 to 100 per cent sterile). On the other hand, inbred 103, a poor pollen producer, had only 60 per cent of its progenies in the highly sterile class. A higher proportion of the 103 crosses remained self-fertile following selection for low sterility. These sub-lines may possess pollen restoring ability.

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# POTATO COMMON SCAB INVESTIGATIONS

## I. A SURVEY OF DISEASE INCIDENCE IN SOUTHERN ONTARIO<sup>1</sup>

J. K. RICHARDSON<sup>2</sup> AND T. J. HEEG<sup>3</sup>

[Received for publication June 10, 1953]

### ABSTRACT

In 1948 and 1949 field surveys were conducted in the more important potato growing areas in southern Ontario in an attempt to correlate crop sequences, cultural practices, and various soil factors with the incidence of common scab in potatoes. The disease in the surveyed fields varied from less than one to over 33 per cent and the type of infection from deep pitted lesions to superficial scurfy areas. The crop preceding potatoes in the rotation appeared to have some influence on the incidence of scab. Data showed a relationship between scab incidence and pH, with low scab associated with values below 5.5. There did not appear to be any relation between the incidence of scab and either the texture of the soil or the amount of chemical fertilizer applied to the potato crop. In 1948, there seemed to be a negative relationship between the incidence of scab and the percentage organic matter and total nitrogen in the soil when eight counties were surveyed and the results considered collectively, but in 1949 when the survey was confined to South Simcoe County the relationship was reversed.

### INTRODUCTION

A survey of the potato growing districts of southern Ontario was undertaken during 1948 and 1949 in order to ascertain (1) the severity of potato common scab caused by *Streptomyces scabies* (Thaxter) Waksman and Henrici, and (2) possible association between disease incidence, crop sequence and chemical and physical properties of the soil. It has been recognized that soils with low pH produce potatoes of low scab incidence and that relatively high moisture levels also favour this condition.

In the spring of 1948, 44 growers in the counties of Dufferin, Durham, Middlesex, Peel, North Simcoe, South Simcoe, Wellington, and Wentworth, who claimed to have a severe scab problem, supplied data on 82 different fields where potatoes were to be grown. In 1949, additional surveys were conducted, first, on 56 fields in the Alliston area where the effect of different rotation schedules could be observed under uniform environmental conditions; and, second, on 18 fields in different districts where soybeans had been grown as a cover crop the previous year. The effect of this crop under field conditions was checked because it had reduced the incidence of scab in greenhouse experiments.

In order to keep all data as comparable as possible and to minimize possible variations in large acreages, records were taken from two-acre areas in each field. The soil texture was recorded and both physical and chemical analyses of the soil in each field were made in the spring before planting and again when the potatoes were dug. Information was also gathered on drainage, crop sequences, cultural practices, and treatments applied to the potato crop.

<sup>1</sup> Contribution from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada—Contribution No. 1263—and the Soils Department, Ontario Agricultural College, Guelph, Ontario; an investigation carried on as part of the potato scab research project conducted under the Ontario Potato Scab Research Committee.

<sup>2</sup> Associate Plant Pathologist, Laboratory of Plant Pathology, St. Catharines, Ontario.

<sup>3</sup> Associate Professor, Department of Soils, Ontario Agricultural College, Guelph, Ontario.



At harvest scab readings were taken on a representative sample of 100 tubers from 25 hills selected at random from each area surveyed. The percentage of the surface of each tuber infected by scab was estimated with the aid of a series of mathematically accurate charts (Figure 1) and the average recorded as the scab incidence of the field.

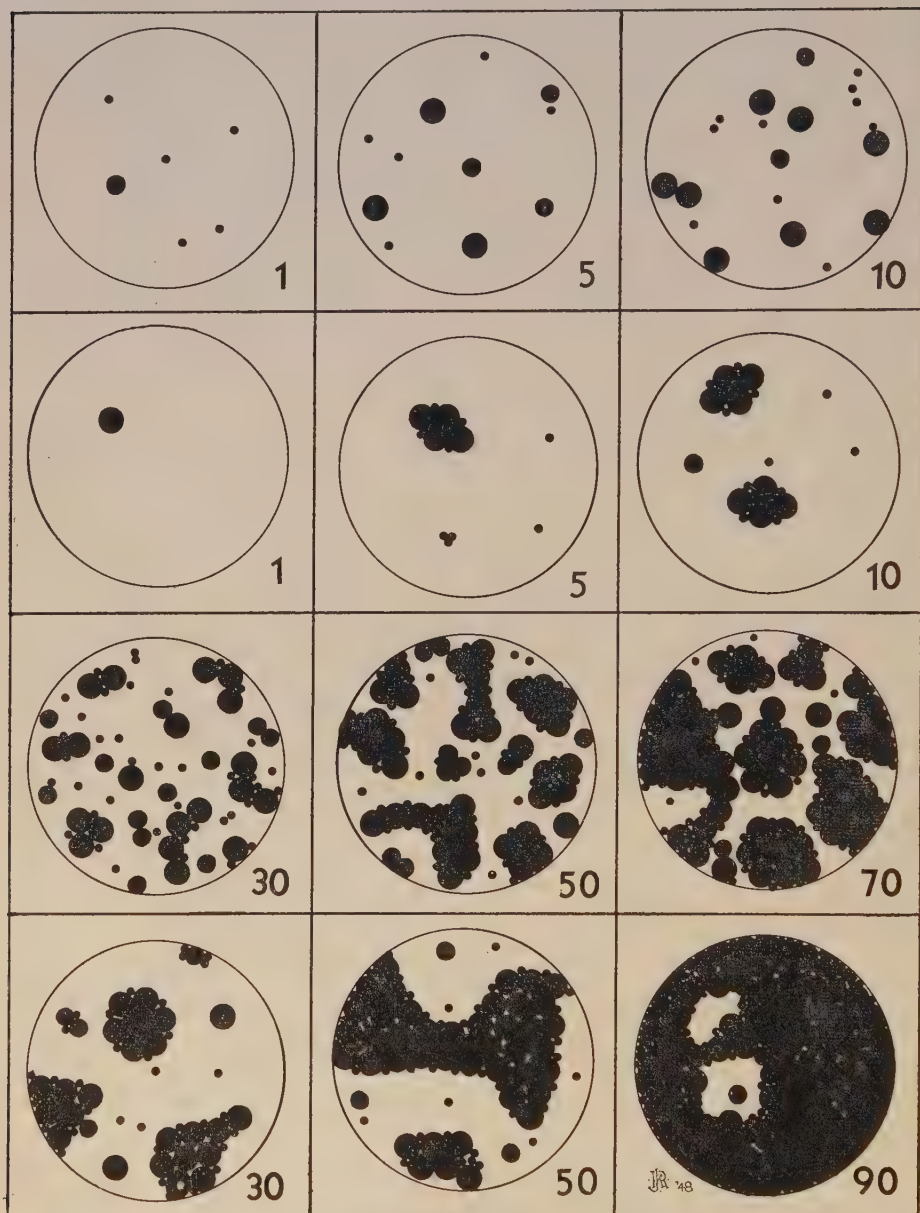


FIGURE 1.

## RESULTS AND DISCUSSION

### *General*

Tuber samples collected from the surveyed fields showed an average scab incidence varying from less than  $\frac{1}{2}$  to more than 33 per cent. The type of infection varied from deep pitted lesions to scattered scurfy areas, but whether this variability was due to differences in environmental conditions or to strains of the pathogen was not determined.

In general, most of the scab found in a single field conformed to one type although the amount varied from hill to hill and even from tuber to tuber. It was also noted that within a district a specific type of lesion was predominant. In the Barrie, South Simcoe, Dufferin, and Wellington areas the scab was severe, often of the deep pitted type, whereas in Durham and Middlesex counties and the Lafontaine district of North Simcoe County the less severe scurfy type was most common.

The soils in all fields surveyed varied in texture from loams to sandy loams, the textural classes most common in commercial potato-growing areas. Incidence of scab was not associated with variations in the texture of the soil.

The amount of chemical fertilizers applied to potato fields either in the fall preceding planting, at planting time, or both, varied from 450 lb. to 3200 lb. per acre but there was no consistent variation between the quantity applied and the incidence of scab in the crop that was produced.

Eight different varieties of potatoes were grown in the fields surveyed the first year, but only Katahdin, Irish Cobbler, and Chippewa were in sufficient number to provide comparative data. The Katahdin tubers from 37 fields located in all areas had an average scab incidence of 4.2 per cent and Irish Cobbler tubers from 28 fields in the same localities showed a scab incidence of 4.3 per cent. Although there were only six fields of the Chippewa variety included in the survey, this variety showed a slightly higher scab incidence of 6.4 per cent. In addition, it was also noted that scab lesions were more severe on Chippewa than on either of the other two varieties when grown in the same field.

Crop rotations and cultural practices were so varied that no two fields were identical or completely comparable. However, it was possible to correlate scab incidence with certain data obtained in the soil analyses and with a number of specific factors which were operative in some fields but not in others. In analysing the effects of certain individual factors on scab incidence, variables such as crop rotations, treatments and cultural practices had to be disregarded although it is fully realized that such variables might alter the results appreciably. In spite of the possibility of disturbance by other factors the analyses show some rather definite trends.

### *Crop Sequence*

The variations in crop rotations in the surveyed fields were so wide that it was impossible to make any significant analyses of their effects on scab. However, crops immediately preceding potatoes could be divided into fairly uniform groups, as listed and compared in Table 1.

TABLE 1.—THE INCIDENCE OF SCAB IN POTATOES FOLLOWING VARIOUS CROPS IN A ROTATION

Crop preceding potatoes	Per cent scab	Number of fields
Potatoes	6.2	16
Spring grain	3.3	27
Mixed hay	3.2	23
Fall wheat	3.1	12
Various legumes	2.5	21
Pasture	2.4	15
Soybean (cover crop)	1.6	7
Corn	1.6	3
Peas	1.3	4

The above table includes all fields surveyed during 1948 and 1949 and reveals the wide variation of crops used in rotation with potatoes. Preceding crops grown in fewer than three fields have not been included. These surveys showed that scab was more severe where potatoes were grown for two years in the same field but there was little difference between various grain and hay crops in their effect on the incidence of the disease.

The 1948 survey was conducted in seven counties in southwestern Ontario while similar data in 1949 were obtained from a single locality, the Alliston district of South Simcoe County. The effect of a potato crop preceding potatoes was to produce the highest scab incidence in both seasons, but the effect produced by other crops was inconsistent. This could have been due to variations in the soil moisture level during critical periods of tuber infection since unpublished data obtained at St. Catharines have shown that scab development is reduced under high soil moisture conditions.

All soybean cover crops were more or less weedy and in some cases the amount of soybean material incorporated into the soil was very little in excess of the other mixed vegetation. Even so it is evident that scab incidence in potatoes following soybeans was in the lowest range.

#### *Soil Analysis and Scab Incidence*

Table 2 reveals that high scab is associated with pH values between 6.0 and 7.0 when all varieties are considered. The peak varied in the two years. In 1948 the highest scab occurred between 6.0 and 6.5, whereas in 1949 it was between 6.5 and 7.0. The per cent scab was low in both years in soils having pH values above 7.0. The minimum scab in both years was associated with soils having pH values below 5.5.

When the Katahdin variety is considered alone the same general trend is noted. The 1949 figures indicate that scab percentages decrease with decreasing pH values whereas in 1948 there is a definite peak in percentage scab in the pH range 6.0–6.5.

The amount of organic matter in the various soils was extremely variable, ranging from a low of 1.4 per cent to a high of 5.2 per cent (Table 3). The fields in Dufferin County had the highest average, 4.3 per cent, the adjacent county of South Simcoe was next with 3.6. Durham, North Simcoe, Middlesex, and Wellington averaged 3.1 to 3.3 per cent and Wentworth only 1.9 per cent. The low organic matter content of fields



TABLE 2.—ASSOCIATION BETWEEN POTATO SCAB AND SOIL REACTION

pH range	All varieties					
	1948 survey			1949 survey		
	Number of fields	Average		Number of fields	Average	
		pH	Per cent scab		pH	Per cent scab
7.0 and above	11	7.3	2.6	6	7.3	2.8
6.9-6.5	20	6.8	3.8	9	6.7	5.4
6.4-6.0	13	6.3	8.8	20	6.2	2.1
5.9-5.5	19	5.7	4.7	30	5.7	1.7
5.4 and below	8	5.2	1.6	6	5.3	1.2
Katahdins only						
7.0 and above	5	7.2	1.9	5	7.3	3.4
6.9-6.5	9	6.8	2.3	7	6.8	3.1
6.4-6.0	7	6.3	12.2	19	6.2	2.0
5.9-5.5	9	5.7	2.4	23	5.7	1.6
5.4 and below	4	5.3	1.9	4	5.4	0.9

TABLE 3.—ASSOCIATION BETWEEN POTATO SCAB AND ORGANIC MATTER OF THE SOIL

Organic matter range	All varieties					
	1948 survey			1949 survey		
	Number of fields	Average		Number of fields	Average	
		Per cent O.M.	Per cent scab		Per cent O.M.	Per cent scab
4.50 and above	10	4.8	2.2	7	4.7	7.0
4.49-4.00	5	4.2	1.7	6	4.2	2.6
3.99-3.50	11	3.7	2.8	5	3.7	1.0
3.49-3.00	18	3.3	4.9	16	3.2	1.8
2.99-2.50	12	2.8	5.5	20	2.7	2.0
2.49-2.00	9	2.2	10.3	13	2.4	1.7
1.99 and below	5	1.7	4.8	3	1.6	0.7

in Wentworth County can probably be explained by the fact that hoed crops are usually grown for several years in succession, in one case for 23 years, whereas, in the other areas surveyed, crop rotation is the general practice. Although it was noted that the average incidence of scab in the fields in Wentworth was 9.4 per cent, the highest of any of the counties, the lowest scab (0.3 per cent) was also found in the same county in a field with an organic matter content of 1.9 per cent. Dufferin County fields showed a consistent negative relationship between scab incidence and organic matter content but none of the other potato growing areas revealed a general trend in this direction. Fields that received an application of manure, either in the fall or spring, immediately preceding the potato crop, had a slightly higher organic matter content than those that had received none, 3.6 and 2.9 per cent respectively. The manured fields also had a lower scab incidence, 3.5 compared with 5.9 per cent in non-manured fields.

TABLE 4.—ASSOCIATION BETWEEN POTATO SCAB AND TOTAL NITROGEN OF THE SOIL

Total nitrogen range	All varieties					
	1948 survey			1949 survey		
	Number of fields	Average		Number of fields	Average	
		Per cent N.	Per cent scab		Per cent N.	Per cent scab
0.20 and above	10	0.26	2.3	10	0.22	6.4
0.199-0.150	7	0.17	6.6	20	0.17	1.6
0.149-0.100	10	0.12	8.0	28	0.13	1.7
0.099 and below	3	0.09	2.4	2	0.08	1.0

TABLE 5.—ASSOCIATION BETWEEN POTATO SCAB AND CARBON-NITROGEN RATIO OF THE SOIL

C/N ratio range	All varieties					
	1948 survey			1949 survey		
	Number of fields	Average		Number of fields	Average	
		C/N ratio	Per cent scab		C/N ratio	Per cent scab
14.00/1 and above	—	—	—	3	14.4	0.8
13.99-13.00	2	13.6	4.1	9	13.3	1.3
12.99-12.50	2	12.6	5.0	8	12.7	2.2
12.49-12.00	6	12.2	6.8	15	12.2	1.4
11.99-11.50	2	11.8	8.6	10	11.7	1.7
11.49-11.00	1	11.2	5.8	14	11.2	1.7
10.99-10.50	5	10.6	1.6	9	10.8	2.5
10.49-10.00	2	10.3	4.4	2	10.3	1.9
9.99 and below	10	9.0	4.7	1	5.8	3.3

The relationship between the incidence of scab and organic matter differed between years. In 1948 it was negative, scab increasing as the organic matter decreased, while in 1949 the association was reversed.

The association of total nitrogen with scab was similar to the organic matter picture in that it was reversed in 1949 from what it was in 1948 (Tables 3, 4 and 5). There did not appear to be any definite relation between scab and carbon-nitrogen ratio.

# SELF-INCOMPATIBILITY IN GREEN SPROUTING BROCCOLI (*Brassica oleracea* L. Var. *italica* Plenck)

## I. ITS OCCURRENCE AND POSSIBLE USE IN A BREEDING PROGRAM

T. H. ANSTEY<sup>1</sup>

*Experimental Farm, Agassiz, B.C.*

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### ABSTRACT

A test for self-incompatibility in green sprouting broccoli was made on a random sample of 175 plants. Although no clear distinction could be made with certain plants, arbitrary limits were set. Of the individuals tested, it was found that 51.8 per cent were self-incompatible, 29.6 per cent self-compatible and the remaining 18.6 per cent of the plants fell between these two extremes. Selfed populations from self-incompatible plants produced an average of 0.46 seeds per silique when grown in isolation, while in a crossing block they produced 7.32 seeds per silique. Top cross populations produced from lines inbred for 2 and 4 generations gave an average of 24.2 per cent greater yield than a recommended commercial variety.

### INTRODUCTION

Green sprouting broccoli has become an important crop on the west coast of Canada within the past five years. Plants from all commercial seed lots tested have shown variation with respect to date of maturity, type and size of curd, as well as other characteristics.

One method of improving commercial varieties of broccoli might be the utilization of  $F_1$  hybrids. Kakizaki (4) in 1926 showed that cabbage contained genes causing self-incompatibility of a type similar to those demonstrated by East and Mangelsdorf (2) in *Nicotiana*. In 1932 Pearson (7) suggested the use of self-incompatibility in cabbage for the production of a commercial  $F_1$  hybrid. Sears (9) reported on self-incompatibility in sprouting broccoli and Pearson (8) described a method of producing  $F_1$  hybrid broccoli seed using caged plants and bees. More recently Odland and Noll (6) and Attia and Munger (1) have expanded on this method with cabbage.

Freeman (3) and Moore and Allmendinger (5) have recently tested many commercial varieties of sprouting broccoli under west coast conditions and have found Waltham 29 to be a variety of good quality and high yield.

The present paper reports the frequency with which self-incompatible green sprouting broccoli plants occur, the feasibility of producing  $F_1$  hybrid seed by the utilization of self-incompatible cross-compatible lines, and the expression of positive heterosis in the top cross when determined by total marketable production.

### MATERIALS AND METHODS

#### *Frequency of Self-Incompatibility*

Cuttings from selected broccoli plants were made and grown in an insect-free greenhouse. When blooming commenced, the flowers were selfed at anthesis and in bud, on at least three racemes per plant. This

<sup>1</sup> Senior Horticulturist; now Superintendent, Experimental Station, Summerland, B.C.



manipulation consisted of exposing stigmas of flowers in the bud stage and selfing with pollen from flowers at anthesis on the same plant. Flowers at anthesis were selfed with their own pollen. The number of seeds produced per silique was recorded in order on the raceme. To compare the degree of self-incompatibility of different plants, the data were converted to a ratio of the number of seeds per silique when selfed in the bud, to the number when selfed at anthesis. Thus, a plant producing as many seeds per silique when selfed in the bud as when selfed at anthesis would have a ratio of 1 : 1. If more seeds were produced from bud pollinations than from anthesis pollinations, the ratio would be greater than 1 : 1. Theoretically a ratio of 1 : 1 would indicate a self-compatible plant, while a ratio greater than 1 : 1 would indicate a self-incompatible plant. In this study, however, plants with a compatibility ratio less than 2.0 : 1 were considered to be self-compatible, while those with a compatibility ratio greater than 9.9 : 1 were considered to be self-incompatible. Those plants with ratios between 2.0 : 1 and 9.9 : 1 were considered doubtful cases. If a plant produced abnormally few seeds in the bud and an equal number of seeds at anthesis giving a compatibility ratio of 1 : 1, it should not be classified as self-compatible. Plants in this study giving seed yields from bud pollinations less than the mean for a normal seed set minus one standard deviation were discarded. To obtain a standard for normal seed set, a sample of siliques from a commercial broccoli seed plot was taken and the number of seeds per silique determined.

Over a period of three years, 175 plants in the broccoli breeding program have been tested for self-incompatibility. The plants tested were selections from commercial varieties and foundation breeding stock. No selection had been made for self-incompatibility and the plants could therefore be considered a random sample with respect to this characteristic.

#### *Production of $F_1$ Hybrid Seed*

Populations were grown from selfed seed obtained by bud pollination of eight self-incompatible plants. These plants were unrelated as far as was known, being selected from the following commercial seed stocks:

Plant number	Variety and seed source
33	Early One, Eastern States Farmers' Exchange
36	Early, Ferry Morse
55, 58, 59, 61, 62, 70	Medium, Ferry Morse

Each of the eight populations was set in an individual block, isolated by at least one mile. These will be called the "isolation blocks". Five similar plants from each of the same eight populations were set out in a single block which will be called the "crossing block". Five racemes were taken at random from each plant in all blocks and the number of seeds in five adjacent siliques on each raceme was counted. A comparison between the

average number of seeds per silique for populations under each set of conditions would indicate their self- and cross-compatibility under natural conditions. All plants upon which data were taken bloomed freely and set seed naturally.

### *Expression of Heterosis in the $F_1$*

Top cross seed from seven self-incompatible broccoli selections inbred for two to four generations were produced in the greenhouse. The male top cross parents consisted of two plants selected from a polycross population showing desirable plant characteristics. They were thought to represent as wide a source of germ plasma as was available since the 10 parents in the polycross were unrelated. Thus 14 top cross and 7 self populations were compared with the standard recommended variety—Waltham 29. Seed was produced under greenhouse conditions and the supply was limited. The experiment was therefore laid out in a randomized paired plot design, each plot containing sub-plots of selfed, two top cross, and commercial variety populations. No replication was possible except for method of crossing.

## RESULTS AND DISCUSSION

### *Frequency of Self-Incompatibility*

The normal seed set per silique on a commercial seed crop was obtained by counting the seeds in each of 5 siliques gathered at random from 330 plants. The mean seed set for these 1650 siliques was  $7.91 \pm 3.74$  seeds. Therefore, plants in the self-incompatibility study having fewer than 4.17 seeds per silique when selfed in the bud were considered to have abnormally low set and were omitted from the study. Of the 175 plants tested for self-compatibility, 13 produced less than 4.17 seeds per silique when selfed in the bud. The frequency distribution of the remaining 162 plants in each of 7 compatibility ratio groups is presented in Table 1. Of the 84 plants with a self-compatibility ratio of 10.0 : 1 or greater, 37 produced no seed when selfed at anthesis and therefore had a self-compatibility ratio of  $\alpha : 1$ . It is noted that 29.6 per cent of the plants tested were self-compatible (compatibility ratios of 1.9 : 1 or less), while 51.8 per cent of the plants were completely or almost completely self-incompatible (compatibility ratio of 10.0 : 1 or greater). The remaining 18.6 per cent of the plants tested fell between these extremes.

TABLE 1.—THE FREQUENCY DISTRIBUTION OF GREEN SPROUTING BROCCOLI PLANTS CLASSIFIED IN VARIOUS SELF-COMPATIBILITY RATIO\* GROUPS

Frequency distribution as:	Compatibility ratios							Total
	10.0 : 1 and greater	8.0 : 1 to 9.9 : 1	6.0 : 1 to 7.9 : 1	4.0 : 1 to 5.9 : 1	2.0 : 1 to 3.9 : 1	1.0 : 1 to 1.9 : 1	1.0 : 1 and less	
Numbers	84	4	4	7	15	42	6	162
Percentage	51.8	2.5	2.5	4.3	9.3	25.9	3.7	100.0

\* The compatibility ratio is the ratio of the number of seeds per silique as a result of selfing in the bud to the number of seeds per silique as a result of selfing at anthesis provided that more than 4.17 seeds per silique are produced in the bud.

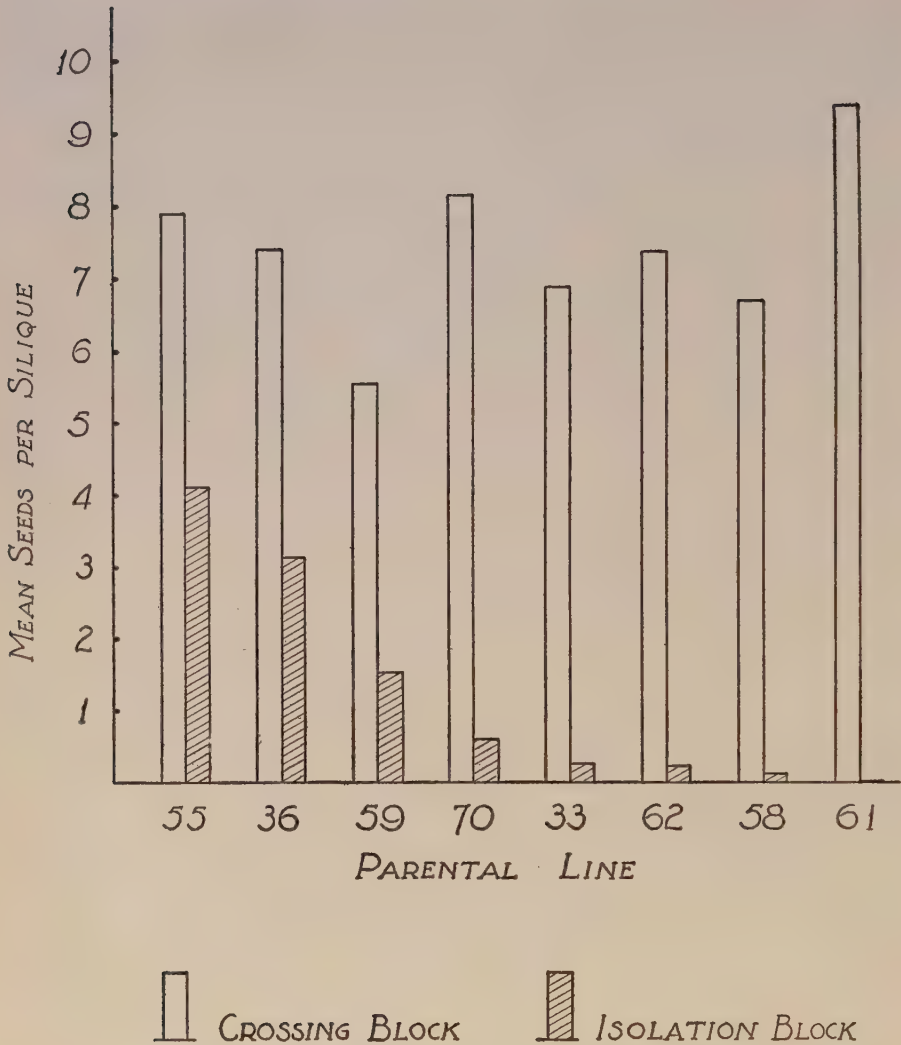


FIGURE 1. A comparison of seed counts from eight self-incompatible lines when grown in crossing and in isolation blocks.

#### *Production of $F_1$ Hybrid Seed*

Seed counts from crossing and isolation blocks are presented in Figure 1. Lines 55 and 36 were sib-compatible although their seed count from the isolation blocks was below that found in the crossing block. The remaining six selfed lines proved to be nearly or completely sib-incompatible and it will be seen from Figure 1 that they produced on an average 0.46 seeds per silique while, when in a crossing block, they produced 7.32 seeds per silique or 15.9 times as many seeds as when isolated. The total seed set in the crossing block averaged 5.5 times that obtained in the isolation blocks. Therefore, much of the seed produced in the crossing block must have resulted from crossing rather than from selfing or sibbing.



TABLE 2.—MEAN YIELD IN TONS PER ACRE OF SELFED, TOP CROSSED, AND COMMERCIAL VARIETY OF GREEN SPROUTING BROCCOLI

Population	Mean yield	Increase or decrease from commercial variety
	T/A	%
Selfed	4.81	— 9.9
Top Crossed	6.63	+ 24.2
Commercial Variety (Waltham 29)	5.34	—
L.S.D. (P = 0.05)	0.94	—

*Expression of Heterosis in the F<sub>1</sub>*

Yields from the top cross studies are presented in Table 2. Because of drought conditions at one end of the field, yields of Waltham 29 were extremely variable. However, since the experiment was a randomized paired plot design, yields of the selfed and top cross populations were in each case directly compared with the corresponding yield of Waltham 29. It will be noted that the general mean yield of the top cross population is higher by 24.2 per cent than the mean yield of the commercial variety, while the mean yield of the selfed populations is 9.9 per cent lower than the yield of the commercial variety. It would appear that greater yields of broccoli than are at present being obtained can be expected from certain hybrid populations.

## CONCLUSIONS

The occurrence of self-incompatibility in green sprouting broccoli has been confirmed. In a random population of green sprouting broccoli plants about 50 per cent of the individuals were almost or completely self-incompatible, 30 per cent were self-compatible while the remaining 20 per cent of the individuals were intermediate with respect to self-incompatibility. Self-incompatibility in broccoli may be used in the production of hybrid seed by growing self-incompatible, cross-compatible lines in close proximity. It has been demonstrated by the use of the top cross test that increased yields from hybrid broccoli may be expected in some cases.

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# THE USE OF THE TRIETHANOLAMINE SALT OF 2,4,5-TRICHLOROPHENOXY-PROPIONIC ACID (COLOR-SET 1004) AS A STOP-DROP SPRAY FOR APPLES<sup>1</sup>

D. V. FISHER<sup>2</sup> AND S. W. PORRITT<sup>3</sup>

*Experimental Station, Summerland, B.C.*

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## ABSTRACT

The triethanolamine salt of 2,4,5-TP (Color-Set 1004) was applied at the rate of 900 gallons per acre with a concentration of 20 parts per million to McIntosh and Delicious apples in six different orchards. Spray was applied 10 to 15 days ahead of picking with McIntosh, and 28 to 37 days from picking with the Delicious variety. In orchards where dropping occurred, the spray proved highly effective, even under severe windstorm conditions. Respiration determinations, pressure tests and organoleptic evaluations indicated no increase in maturity or ripening in sprayed as compared with check fruit.

Color-Set 1004 is a hormone preparation which has attracted wide attention since its introduction by Dow Chemical Company three years ago. It is the triethanolamine salt of  $\alpha$ -(2,4,5-trichlorophenoxy) propionic acid and has been reported to show promise as a stop-drop spray for apples. Present reports indicate that the superiority of Color-Set 1004 over the standard stop-drop chemical,  $\alpha$ -naphthalene acetic acid, lies in its greater adhesive properties, longer period of effectiveness (3 to 6 weeks) and, in some cases, its tendency to enhance red colour development. It is applied as an aqueous solution at a concentration of not more than 20 parts per million at least 5 days before dropping is expected.

## REVIEW OF LITERATURE

Since the classical work reported in 1939 by Gardner *et al.* (5) on the control of harvest drop of apples by the use of hormone sprays, a great volume of experimental data has been developed by numerous workers in widely separated fruit growing areas. Alpha-naphthalene acetic acid and  $\alpha$ -naphthalene acetamide have proved generally effective for most varieties of apples, while 2,4-dichlorophenoxyacetic acid has shown specific effectiveness with the Winesap group of varieties (2, 3, 9). Under certain climatic conditions and with different orchards, however, consistently favourable results have not always followed the use of  $\alpha$ -naphthalene acetic acid sprays. The search, therefore, has continued for a longer-lasting, more effective stop-drop hormone spray. Thus, 2-methyl,4-chlorophenoxy-acetic acid was suggested by Mitchell *et al.* (10) as an improved stop-drop spray for McIntosh. More recently Edgerton and Hoffman (5) have shown that 2,4,5-trichlorophenoxy-propionic acid is effective as stop-drop for McIntosh and may be applied at least 26 days ahead of  $\alpha$ -naphthalene acetic acid. They also reported some advancement of maturity and red colour development. Further tests conducted by Batjer and Rogers (4) in Washington indicate that 2,4,5,-TP is effective in controlling pre-harvest drop of Delicious

<sup>1</sup> Contribution No. 793-A from the Horticultural Division, Experimental Farms Service.

<sup>2</sup> Senior Horticulturist in charge of Fruit Harvesting and Storage Investigations.

<sup>3</sup> Horticulturist.



and Winesap apples when applied as much as a month or more ahead of harvest at 48 to 96 grams per acre. No increase in red colour was observed in test plots. Nelson and Blair (11) noted that 2,4,5-TP was especially effective on early varieties such as Melba for both drop control and colour improvement, but not quite so effective with McIntosh.

The physiological effect on fruit of hormone sprays has received careful scrutiny. It is generally conceded that, under certain conditions and with certain varieties,  $\alpha$ -naphthalene acetic acid and 2,4,-D may accelerate rate of respiration and ripening (1, 7, 8, 13, 14), although with certain kinds of detached fruits Gerhardt *et al.* (8) found no significant physiological or physical response to these chemicals. Reports have been conflicting with respect to the maturity effects of 2,4,5-TP. Batjer and Rogers (4) believe no adverse affects are likely to follow the application of 2,4,5-TP under central Washington conditions. On the other hand, Smith (12) reports marked acceleration of maturity following the use of 2,4,5-TP, so much so, that fruit on trees sprayed 2 to 3 weeks before harvest was ripe, when picked.

### EXPERIMENTAL METHODS AND RESULTS

#### Orchard Studies

Color-Set 1004 was tested in 1951 under Okanagan conditions in six McIntosh and three Delicious orchards. The material was applied in the period of September 7 to 9. Conventional gun-type sprayers were used except in the Butler orchard, East Kelowna, where a concentrate sprayer

TABLE 1.—INFLUENCE OF COLOR-SET 1004\* ON AMOUNT OF FRUIT DROP ON MCINTOSH AND DELICIOUS APPLES

Grower	Date of application	Days between application and harvest	Apple drop per tree		Remarks
			Color-Set 1004	Check	
			boxes	boxes	
<i>McIntosh</i> —					
Coldstream Ranch	Sept. 6	10	0	2.0	
Lakeview Orchards	Sept. 7	15	0	4.5	Definite colour improvement.
T. S. Towgood	Sept. 9	13	0	3.0	Effective 2 weeks only.
L. E. Marshall	Sept. 9	11	0	0	Picked before severe wind.
L. G. Butler*	Sept. 7	14	2.5	4.5	
A. G. Des Brisay	Sept. 9	11	0.25	0.25	Picked before severe wind.
<i>Delicious</i> —					
Coldstream Ranch	Sept. 6	37	0	2.5	
L. E. Marshall	Sept. 9	28	0	0	
A. G. Des Brisay	Sept. 9	32	0	2.0	

\* The material was applied at 20 parts per million with a hand gun sprayer except on the Butler orchard where it was applied with a concentrate sprayer at 200 parts per million.

was employed, using ten times dilute concentration. Each grower was supplied with enough material to spray 8 good-size trees with 20 gallons of dilute spray per tree.

At picking-time records were made of dropped fruit under sprayed and control trees as indicated in Table 1. The fruit was shipped immediately to the Experimental Station, where it was pressure-tested, samples removed for respiration determination, and the balance placed in 31° F. cold storage for later examination. Data on drop control are presented in Table 1.

These data indicate the 2,4,5-TP was highly effective as a stop-drop spray. It should be noted, however, that on the Marshall orchard no dropping occurred even on the check trees.

Only one orchardist (Lakeview Orchards) reported an improvement in colour with the McIntosh variety. No colour improvement was noted with Delicious.

Several growers noted that Color-Set 1004 sprays had the effect of delaying normal abscission of leaves. In fact, in January some trees still were holding their leaves.

#### *Respiration Studies with Fruit from Sprayed Trees*

Since hormone-type materials can cause marked physiological stimulation, the effect of Color-Set upon respiration rate of apples was studied. Respiration determinations were carried out at 65° F. for two to three weeks following picking. Experimental samples consisted of about 30 apples weighing 4 kilograms. These apples were placed in 5-gallon containers and aerated continuously for a day at 6 litres per hour to permit equilibration of gases and temperature. On each succeeding day carbon dioxide output was determined by absorption in 0.1 N barium hydroxide solution in Truog towers, followed by titration with 0.1 N HCl. Carbon dioxide was calculated as milligrams per kilogram of apples per hour. The data are presented in Table 2.

TABLE 2.—INFLUENCE OF COLOR-SET 1004 ON RESPIRATION RATE OF MCINTOSH APPLES AT 65° F. IMMEDIATELY FOLLOWING HARVEST

Grower	Number of daily determinations	Respiration rate at 65° F. Mg. CO <sub>2</sub> per kilogram of apples per hour			
		Color-Set 1004		Check	
		Average	Range	Average	Range
<i>McIntosh—</i>					
Coldstream Ranch	13	22.8	21.3-23.9	23.1	21.7-24.8
L. G. Butler	10	22.3	20.1-24.4	21.5	20.0-24.2
T. S. Towgood	6	24.3	22.6-25.8	24.2	23.3-26.2
A. G. Des Brisay	12	23.5	20.8-26.2	24.3	20.8-26.2
<i>Delicious—</i>					
A. G. Des Brisay	8	20.7	16.9-24.4	19.5	13.8-24.3
2nd A. G. Des Brisay*	8	18.3	14.0-21.0	19.5	13.8-24.3
L. E. Marshall	7	20.2	18.3-22.2	19.2	15.2-23.3

\* "Parmone"  $\alpha$ -naphthalene acetic acid 10 p.p.m.

These data indicate that no significant differences in rate of respiration were detected between sprayed and unsprayed fruit in either McIntosh or Delicious. It will be noted that fruit from trees sprayed with Parmone ( $\alpha$ -naphthalene acetic acid) was included in one of the Delicious plots. For some reason, this fruit displayed a lower respiration rate than either the check sample or the fruit from trees sprayed with Color-Set. As far as appearance was concerned, no differences in maturity were found between check and sprayed apples from any orchard.

### *Pressure Tests and Eating Quality*

Apples from each lot were pressure tested when placed in cold storage. On January 25 the fruit was taken from cold storage and placed in the ripening room at 65° F. where it was examined 10 days later. The data for the two examinations are included in Table 3.

TABLE 3.—FIRMNESS AND QUALITY OF SPRAYED AND CHECK APPLES AT PICKING AND AFTER COLD STORAGE

Grower	Firmness of fruit at harvest		Firmness of fruit after storage		Eating quality of fruit after storage	
	Color-Set	Check	Color-Set	Check	Color-Set	Check
	lb.	lb.	lb.	lb.		
<i>McIntosh</i> —						
Coldstream Ranch	15.2	15.2	10.7	10.5	Fair	Fair
Lakeview Orchards	13.1	14.2	9.4	9.8	Fair-Poor	Fair-Poor
T. S. Towgood	14.6	14.8	10.7	10.7	Fair	Fair
L. E. Marshall	12.8	13.8	9.3	9.7	Fair	Fair
L. G. Butler	14.8	15.5	10.5	10.5	Fair-Good	Fair-Good
A. G. Des Brisay	14.3	14.8	10.8	11.2	Fair	Fair
<i>Delicious</i> —						
Coldstream Ranch	Data not taken		11.9	13.1	Poor	Poor
L. E. Marshall			12.3	12.5	Fair	Fair
A. G. Des Brisay			14.1	12.0	Fair	Fair

These data indicate slight but inconsistent differences in firmness between check and sprayed fruit. Likewise, no differences in eating quality were found between sprayed and control fruit.

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# THE EFFECTS OF THE SHEEP KED (*MELOPHAGUS OVINUS* L.) ON FEEDER LAMBS<sup>1</sup>

F. WHITING, W. A. NELSON, S. B. SLEN AND L. M. BEZEAU<sup>2</sup>

*Experimental Station and Laboratory of Entomology, Lethbridge, Alta.*

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## ABSTRACT

Experiments were carried out during a 3-year period (1950-1953) to determine the effect of sheep ked (*Melophagus ovinus* L.) infestation upon body weight gains and wool production of feeder lambs and to determine the influence of the level of nutrition of the sheep upon ked population. There were no significant differences in body weight gains or wool production between ked-free and ked-infested feeder lambs. The ked populations on poorly-fed lambs increased more rapidly and became larger than those on well-fed lambs. The ked population on both well-fed and poorly-fed lambs increased during the first 2 to 3 months on feed and then declined. Preliminary information indicates that the ked population on pregnant ewes continues to increase throughout the winter and spring months. No relationship was found between the number of keds on a sheep and certain blood constituents or the suint and fat content of the fleece.

It is a general opinion among lamb feeders that heavy infestations of sheep keds (*Melophagus ovinus* L.) reduce body weight gains of feeder lambs. Also it has been observed that sheep in good condition rarely support heavy infestations of keds, while those in poor condition often are heavily infested. Whether ked infestation is the cause or the result of poor condition in sheep has not been established satisfactorily. Therefore, it is important to know the extent to which ked infestation does affect gains in the feedlot so that the feeder can judge whether it is economically sound to spray or dust heavily infested lambs.

Since this investigation was undertaken, three papers have appeared in the scientific literature reporting the results of experiments very similar to those reported here (2, 6, and 9).

Bosman *et al.* (2) conducted studies using mainly yearling and 2-year-old Merino wethers in which some were infested and others kept free from keds. They concluded from these studies that, given a good standard of feeding, ked infestation did not adversely affect gains in weight or the production of scoured wool. However, given a limited amount of feed or a poor ration, ked infestation had an injurious effect on the health of sheep but did not influence the amount of scoured wool produced. Also, they reported that well-fed sheep did not become infested readily.

Muma *et al.* (6) reported from studies on feeder lambs that weight-gain differences between infested and non-infested sheep could not be demonstrated. Pfadt *et al.* (9) also found no significant differences in weight gains between ked-controlled and non-ked-controlled feeder lambs. They reported also that, although ked infestation was moderate to heavy on the lambs at the start of the trial, ked numbers on the untreated lots declined after the lambs had been on feed several weeks.

<sup>1</sup> Contribution from the Division of Animal Husbandry, Experimental Farms Service, and the Division of Entomology, Science Service, Canada Department of Agriculture, Ottawa.

<sup>2</sup> Animal Nutritionist, Assistant Entomologist, Wool Specialist, and Assistant Animal Nutritionist, respectively.

McKenna and Fearn (5) reported that the degree of infestation depended upon the body condition of the sheep and that there was a seasonal rise and fall in the number of keds on the sheep. Also, they noted that the ked population on heavily-infested sheep rapidly declined when they were grazed on good pasture.

Imes (3) reported that ked-infested sheep had reduced vitality, produced less wool, and ate less feed. Schwardt and Matthysse (10) stated that infested lambs gained weight slowly and probably were more susceptible to disease and to infestation by internal parasites.

In order to study the influence of ked infestation on feeder lambs during the fattening period and to study the extent to which level of nutrition influences ked populations, experiments were undertaken with feeder lambs at the Experimental Station, Lethbridge, Alberta, during the winter feeding periods of 1950-51, 1951-52, and 1952-53.

#### PROCEDURE

In the fall of 1950, 18 uniform wether lambs of Suffolk  $\times$  Rambouillet breeding (selected from a larger group) were divided into three lots of 6 lambs each on the basis of body weight and ked infestation. These lambs had not been dipped or sprayed during the previous summer and were infested quite heavily with keds. Lot 1 was made ked-free by picking all keds off the lambs and then thoroughly dusting the lambs with an insecticide containing rotenone. Lots 2 and 3 remained infested. Lots 1 and 2 were fed individually, and received a ration of grass hay and a concentrate mixture (oats 50 per cent, linseed oil-cake 20 per cent, and dried molasses beet pulp 30 per cent), supplemented with a mineral mixture containing bonemeal, salt, cobalt, and copper, and with a vitamin A supplement (3000 I.U. of vitamin A daily per lamb). Lot 3 was group fed a ration of oat straw and dried molasses beet pulp without mineral or vitamin supplementation. The lambs were weighed at 28-day intervals during the experiment. The keds on the lambs were counted at approximately 28-day intervals using the 5-minute-count method as outlined by Nelson and Slen (8). This method counts approximately 20 per cent of the total number of keds on the lamb (8).

In the fall of 1951 the experiment was repeated using three lots of 6 crossbred lambs of Rambouillet  $\times$  Canadian Corriedale breeding. As in the previous year the lambs in Lots 1 and 2 were individually fed. The ration fed during 1951-52 consisted of alfalfa hay and a concentrate mixture (oats 30 per cent, barley 40 per cent, linseed oilcake 10 per cent, dried molasses beet pulp 20 per cent), with the same mineral and vitamin supplements as fed during 1950-51. Lot 3 was fed as in the previous year. Ked counts and body weights were taken as outlined for 1950-51 with the exception that the first ked count was a total-live count rather than a 5-minute count.

The experiment was repeated again in 1952-53 using three lots each with 11 lambs of Rambouillet-Romnelet breeding. The lambs were fed the same rations as during 1951-52 but were group-fed. To ensure a heavier infestation of keds at the beginning of the experiment each lamb in Lots 2 and 3 was infested with 200 additional keds obtained from sheep within the original flock.



TABLE 1.—AVERAGE INITIAL AND FINAL WEIGHTS, AVERAGE DAILY GAINS, AVERAGE DAILY FEED CONSUMPTION, AND FEED CONSUMPTION PER 100 POUNDS BODY GAIN AS AFFECTED BY KED INFESTATION AND RATION

	Lot 1	Lot 2	Lot 3
	Good ration ked free	Good ration ked infested	Poor ration ked infested
<i>1950-51 Experiment</i>			
Number of lambs per lot	6	6	6
Number of days on experiment	137	137	137
Average initial weight (lb.)	88	87	86
Average final weight (lb.)	121	121	80
Average daily gain (lb.)	$0.24 \pm 0.02^1$	$0.25 \pm 0.02$	$-0.05 \pm 0.02$
<i>Average Daily Feed Consumption (lb.) per Lamb—</i>			
Roughage	1.04	1.04	0.8
Concentrate mixture	1.43	1.43	0.7
<i>Average Feed Consumption (lb.) per 100 lb. of Body Gain—</i>			
Roughage	430	421	—
Concentrate mixture	596	581	—
<i>1951-52 Experiment</i>			
Number of lambs per lot	6	5 <sup>2</sup>	6
Number of days on experiment	117	117	117
Average initial weight (lb.)	70	71	70
Average final weight (lb.)	103	104	76
Average daily gain (lb.)	$0.28 \pm 0.04$	$0.28 \pm 0.04$	$0.06 \pm 0.03$
<i>Average Daily Feed Consumption (lb.) per Lamb—</i>			
Roughage	1.08	1.07	1.0
Concentrate mixture	1.30	1.30	1.0
<i>Average Feed Consumption (lb.) per 100 lb. of Body Gain—</i>			
Roughage	381	378	1746
Concentrate mixture	475	479	1746
<i>1952-53 Experiment</i>			
Number of lambs per lot	11	11	11
Number of days on experiment	139	139	139
Average initial weight (lb.)	48	51	50
Average final weight (lb.)	100	98	66
Average daily gain (lb.)	$0.38 \pm 0.05$	$0.34 \pm 0.05$	$0.11 \pm 0.02$
<i>Average Daily Feed Consumption (lb.) per Lamb—</i>			
Roughage	1.41	1.36	1.0
Concentrate mixture	1.48	1.48	1.0
<i>Average Feed Consumption (lb.) per 100 lb. Body Gain—</i>			
Roughage	372	400	865
Concentrate mixture	391	438	865

<sup>1</sup> Standard deviation.

<sup>2</sup> One lamb died from unknown cause(s).

During the 1950-51 experiment blood samples were collected from all lambs after the lambs had been on feed 83 days and 137 days. These samples were analysed for total protein, packed cell volume, hemoglobin, and red and white cell counts.

The lambs were shorn at the end of the 1950-51 experiment to determine raw and clean fleece weights. At the completion of the 1951-52 and 1952-53 experiments, wool samples were taken from a measured area on the side of each lamb. Weight of clean wool, percentage of fat and suint, and yield of clean wool were determined on these samples.

## RESULTS AND DISCUSSION

The average gains and feed efficiency as influenced by ked infestation and plane of nutrition are shown in Table 1. It is evident from the data presented in this table that in none of the three tests did ked infestation significantly affect the gains made by the lambs. There was no significant correlation within the lots between the number of keds on the individual lambs and their gains during the 3 years' study. The efficiency of feed utilization was not influenced by ked infestation when studied within groups (individual feed and gain data) or between groups fed similar rations. Bosman *et al.* (2), Muma *et al.* (6), and Pfadt *et al.* (9) also have reported that ked infestation did not influence the gains made by lambs.

Table 2 shows the influence of plane of nutrition on ked populations. There was a wide variation in the number of keds found on the lambs within groups. Bosman *et al.* (2) and other workers also have reported a wide variation in ked numbers on sheep. Ked populations on the poorly-fed lambs increased more rapidly and became more abundant than on the well-fed lambs. Each year a gradual increase in ked numbers occurred during the first two or three months, but this was followed by a steady decline. Bosman *et al.* (2) and McKenna and Fearn (5) also reported similar findings. Pfadt *et al.* (9) reported a decline in ked population among feeder lambs soon after being put on to feed. The cause of this phenomenon is not known but is being investigated. Pfadt *et al.* (9) and unpublished preliminary data from this Station indicate that the population of keds on pregnant ewes, unlike that on feeder lambs, does not decrease during the early winter months but tends to increase throughout the winter and early spring months.

A study of hemoglobin and plasma protein content, red and white cell counts, and the hematocrit value of the blood taken from the lambs during the 1950-51 experiment showed no significant influence of ked infestation upon these blood constituents. The average values found for Lots 1 and 2 were: white cell count,  $11.3 \times 10^3$  per ccm.; red cell count,  $12.5 \times 10^6$  per ccm.; hemoglobin, 13.4 gm. per 100 ml.; hematocrit, 45.1; and serum protein, 6.7 gm. per 100 ml. Lot 3, presumably due to a lower plane of nutrition, had lower values for all factors measured, ( $9.0 \times 10^3$ ,  $10.8 \times 10^6$ , 11.6, 40.6, and 6.42, respectively). There was no correlation within groups between the number of keds on a lamb and the values for the blood constituents considered above.

TABLE 2.—AVERAGE KED COUNTS AS INFLUENCED BY PLANE OF NUTRITION OF FEEDER LAMBS. (FIVE-MINUTE COUNT EXCEPT WHERE NOTED)<sup>1</sup>

	Lot 1 Good ration ked free	Lot 2 Good ration ked infested	Lot 3 Poor ration ked infested
<i>1950-51 Experiment</i>			
November 2	31 ± 17 <sup>2</sup>	31 ± 20 <sup>2</sup>	32 ± 17 <sup>2</sup>
December 2	—	49 ± 15	42 ± 23
December 28	—	65 ± 27	94 ± 32
January 25	—	38 ± 24	79 ± 31
March 1	—	22 ± 17	47 ± 33
March 22	—	14 ± 9	39 ± 30
<i>1951-52 Experiment</i>			
November 7 (total live count)	70 ± 41	75 ± 38	69 ± 48
December 4	—	65 ± 25	73 ± 36
January 2	—	62 ± 36	83 ± 24
January 31	—	29 ± 33	57 ± 40
February 28	—	13 ± 8	45 ± 27
<i>1952-53 Experiment</i>			
September 27	27 ± 18	27 ± 18	27 ± 15
October 10	—	53 ± 14 <sup>3</sup>	49 ± 8 <sup>3</sup>
November 10	—	62 ± 18	77 ± 15
December 8	—	74 ± 26	93 ± 16
January 8	—	54 ± 42	112 ± 30
February 10	—	12 ± 7	63 ± 25

<sup>1</sup> In the 5-minute-count method approximately 20 per cent of the total ked population is counted (8).<sup>2</sup> Standard deviations.<sup>3</sup> Two hundred keds put on to each lamb in Lots 2 and 3 on October 6.

In Table 3 are shown the raw and clean wool weights taken from a measured area (50 sq. cm.) together with the data on percentage yield of clean wool and percentage fat and suint in the raw wool. There were no significant differences in clean wool production between the ked-free, well-fed lambs (Lot 1) and the ked-infested, well-fed lambs (Lot 2). As would be expected, the poorly-fed lambs in Lot 3 produced less wool than the other two lots. The raw wool from the ked-infested lambs had a lower percentage yield of clean wool than the ked-free lambs. Presumably, this was due to ked excreta and debris. Bosman *et al.* (2) reported similar findings. The suint content of the raw wool from the well-fed, ked-free lot was less (not significantly so) and the fat content higher than that of the wool from the well-fed, ked-infested lot. The poorly-fed, infested lambs had a higher suint content and a lower fat content than the well-fed, infested lambs.

There was no correlation within groups between the number of keds on the individual lambs and the suint or fat content of the wool. Knowles (4) observed that keds destroyed wool fat and McLeod (7) has suggested



TABLE 3.—THE EFFECTS OF KED INFESTATION AND THE NUTRITIONAL STATUS OF LAMBS ON WOOL PRODUCTION (1951-52 AND 1952-53)

	Good ration ked free	Good ration ked infested	Poor ration ked infested
Average weight of raw wool (gm.) <sup>1</sup>	5.4	5.6	5.6
Per cent of yield of clean wool	58.0	56.0	52.0
Average weight of clean wool (gm.)	3.2	3.2	2.2
Per cent of suint in raw wool <sup>2</sup>	21.3	26.3	33.6
Per cent of fat in raw wool <sup>2</sup>	13.6	12.4	10.5

<sup>1</sup> Weight of wool from a 50 sq. cm. area on side of lamb.<sup>2</sup> Based on moisture-free weight of raw wool.

that there may be an inverse relationship between sheep tick (*Ixodes ricinus* L.) infestation and grease content of the wool. A study of the within group data did not indicate any relationship between fat content of the wool and ked population, although group averages indicated that there may be some association. Barker (1) has stated that the amount of fat in the wool may be associated with the level of nutrition of the sheep. If this is so the lower fat content of the wool from Lot 3 may be due to the lower plane of nutrition of this lot of lambs.

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# TRACE ELEMENT CONTENT OF FARMYARD MANURE<sup>1</sup>

H. J. ATKINSON, G. R. GILES AND J. G. DESJARDINS

*Science Service, Canada Department of Agriculture, Ottawa, Canada*

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## ABSTRACT

Forty-four samples of farmyard manure, representing fresh cow, horse, swine, sheep, poultry and mixed manures and composted cow and mixed manures, were analysed for their contents of boron, manganese, cobalt, copper, zinc, molybdenum and ash. Maximum, minimum and average values for the trace elements are presented and the frequency distribution of their concentrations given in the form of histograms. Average values on a dry matter basis were: 20.2 p.p.m. B, 201.1 p.p.m. Mn, 1.04 p.p.m. Co, 15.6 p.p.m. Cu, 96.2 p.p.m. Zn and 2.37 p.p.m. Mo. The correlation coefficients between the element and ash contents were all positive and all except that for cobalt and ash were significant at  $P 0.05$ . On the basis of an acre application of 20 tons of manure containing 80 per cent moisture, the maximum quantities of the trace elements in the manure samples were, with the exception of zinc, somewhat less, and the average quantities considerably less, than the minimum amounts commonly used when treatment is applied as a chemical compound.

## INTRODUCTION

It is known that farmyard manure adds nitrogen, phosphorus and potassium to a soil and has some effect on its physical condition, but other results have been observed from time to time which appeared to be due to some other factor. Several workers (2, 10, 13, 17) have attributed such results to the trace element content. Others have shown that applications of manure have reduced or eliminated disorders due to deficiency of boron (3, 4, 7, 15), zinc (1) and manganese (14).

There are relatively few figures on the trace element content of farmyard manure in the literature. Young (19) found a sample of manure to contain 80 p.p.m. of manganese, 50 p.p.m. of boron and no copper. Steenbjerg (16) showed that Danish farmyard manure contained on the average 55 p.p.m. of manganese, 5.5 p.p.m. of copper and 3.9 p.p.m. of boron. Hester (6) found that dry manure contained 1500 p.p.m. of manganese, 35 p.p.m. of boron and 18 p.p.m. of copper. Other references (5, 8, 11) have given the boron content of manure at about 20 p.p.m. B.

## MATERIALS

Disorders attributable to trace element deficiencies have been reported with increasing frequency in recent years. It was, therefore, considered desirable to obtain information on the occurrence and distribution of trace elements in farmyard manure produced in different parts of Canada. Accordingly, the various Experimental Farms and Stations were requested to obtain samples of manure from their own supplies and from one or more of the farms in the surrounding districts. It was believed that, in this way, a fairly representative group of samples would be obtained.

<sup>1</sup> Scientific Contribution No. 240, Division of Chemistry, Science Service.

Forty-four samples were received from Stations in all provinces and were classed as follows: 18 samples of fresh cow manure; 4 samples of fresh horse manure; 3 samples of fresh swine manure; 1 sample of fresh sheep manure; 1 sample of fresh hen manure; 2 samples of fresh mixed manure; 6 samples of composted cow manure; 9 samples of composted mixed manure. The period of composting varied from a few months to twenty years or more in one case. A few samples were excrement only. Most, however, contained varying amounts of litter, usually straw, although samples from two locations contained wood shavings as well.

### MOISTURE, DRY MATTER AND ASH

As soon as the samples were received, they were dried in a mechanical convection oven for a few days at 60° C., and finally at 105° C. for 24 hours. The dry matter content of the manures ranged from 13.6 to 47.2 per cent.

All samples were finely ground in a Wiley mill. The ash content was determined by ignition in a muffle furnace at 550° C. for 6 hours. The results for fresh manures ranged from 8.2 to 19.9 (av. 11.9) per cent, and those for composted manures from 10.1 to 47.7 (av. 25.7) per cent, of the dry matter. The highest value of the fresh manures was found in a sample of hen manure. The lowest value among the composted materials occurred in a sample which was observed to have a high content of straw and wood shavings. Eight samples of pure feces, which included the hen manure, had an average ash content of 11.9 per cent, the same as for all fresh manures.

A measure of the insoluble ash was obtained by weighing the ignited residue after the sample had been wet-ashed with nitric and perchloric acids, taken up in HCl and filtered. Values ranged from 1.58 to 8.62 per cent in the fresh samples and from 4.41 to 36.39 per cent in the composted samples. In general, the insoluble ash increased as the total ash increased.

### DETERMINATION OF TRACE ELEMENTS

Quantitative determinations were made for six elements: boron, manganese, cobalt, copper, zinc and molybdenum. Boron was determined by ashing the samples in a muffle furnace in the presence of lime and applying the turmeric colour method. For manganese, the sample was ashed in a muffle furnace, treated with HF to remove the silica, then with a drop or two of H<sub>2</sub>SO<sub>4</sub> and taken up in HCl. The periodate procedure was used to develop the colour. The dithizone method was used for cobalt, copper and zinc after samples were wet-ashed with nitric and perchloric acids, treated with HF to remove silica and taken up in HCl. In the above cases, the details of the determinations were essentially those used for soils in these laboratories (18). In the case of molybdenum, after the sample was treated with nitric acid and perchloric acid to destroy organic matter, it was taken up in 5 per cent HCl and the determination made according to the method used in New Jersey (12).



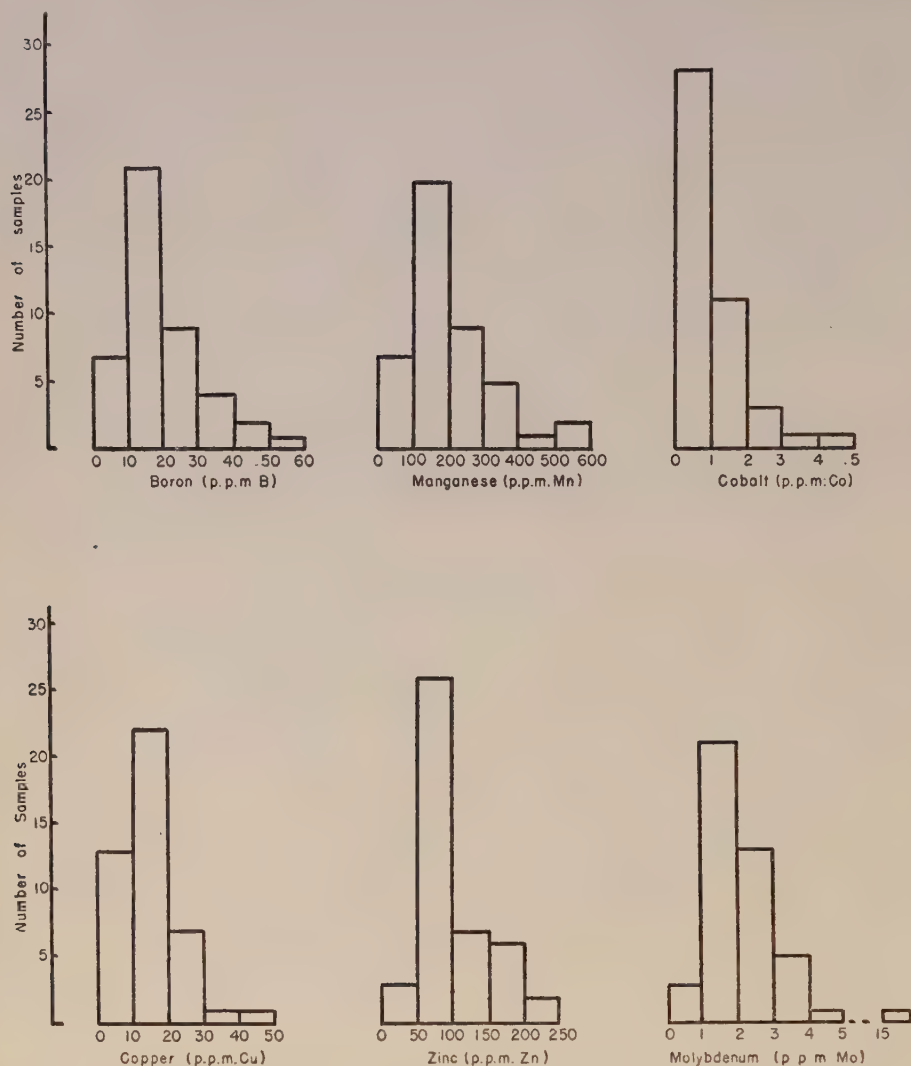


FIGURE 1. Histograms showing frequency distribution of concentrations of trace elements in 44 manure samples.

## RESULTS AND DISCUSSION

Figures showing the minimum, maximum and average values found are presented in Table 1 and the frequency distributions of the concentrations in various ranges are shown as histograms in Figure 1. In general, the results were of the same order as reported in the literature for those elements to which some reference could be found. Manganese and zinc were present in the largest amounts, boron and copper somewhat less and molybdenum and cobalt in lowest concentrations. This order is the same as that given by Mitchell (9) and by Prince (12) for the trace element content of plants.

TABLE 1.—TRACE ELEMENT CONTENT OF MANURES  
(as p.p.m., dry matter basis)

Element	Minimum	Maximum	Average
Boron	4.5	52.0	20.2
Manganese	75.0	549.0	201.1
Cobalt	0.25	4.70	1.04
Copper	7.6	40.8	15.6
Zinc	43.0	247.0	96.2
Molybdenum	0.84	15.83	2.37
Molybdenum*	0.84	4.18	2.06

\* With one exceptionally high value omitted

There is no apparent reason for the extremely high molybdenum content (15.83 p.p.m.) of one sample in comparison with the others. According to information supplied with the sample, it came from animals which had been fed with mineral supplements but there was no similar effect on the other trace elements in this sample nor was the molybdenum content unusually high in other samples where mineral feeding had been practised. The highest value for zinc (247 p.p.m.) was from a sample which was shipped in a metal container and may have become contaminated. The second highest zinc value was 208 p.p.m. The highest and lowest values for cobalt (4.70 and 0.25 p.p.m.) were from fresh cow manures obtained on two farms in the neighbourhood of the Experimental Station at Ste. Anne de la Pocatière, Quebec. Cobalt deficiency disorders have been reported from this area<sup>1</sup> and it may be that a cobalt supplement was fed on the farm where the sample with the high value was obtained. The second highest cobalt value was 3.15 p.p.m.

Correlation coefficients between the ash content of the manures and their content of the trace elements were determined and are presented in Table 2. A very high value ( $r = +0.80$ ) was obtained in the case of boron; values for zinc and molybdenum were just above the level for significance.

On the basis of an acre application of 20 tons of manure containing 80 per cent moisture, the maximum and average amounts of each element that would be added to the soil were calculated and expressed in terms of the chemical compound commonly used in soil treatments. The results,

TABLE 2.—CORRELATION COEFFICIENTS BETWEEN ELEMENT  
AND ASH CONTENTS

Element	Coefficient
Boron	+ 0.80*
Manganese	+ 0.69*
Cobalt	+ 0.27
Copper	+ 0.67*
Zinc	+ 0.44*
Molybdenum	+ 0.40*

\* Significant at P 0.05

<sup>1</sup> Ouellette, G. J. Minor elements in soils and plants of eastern Quebec. *Unpublished*. Paper given at the annual meeting of the Quebec Soils Committee held in Montreal, April 17, 1951.

TABLE 3.—COMPARISON OF ACRE APPLICATIONS OF TRACE ELEMENTS  
IN MANURE AND IN CHEMICALS  
(as pounds of the compound commonly applied to soil)

Element	Compound	In manure*		In chemicals**
		Maximum	Average	
Boron	$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$	3.66	1.41	5 - 40
Manganese	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	17.85	6.53	25 - 50
Cobalt	$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$	0.18	0.04	0.25- 2
Copper	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1.28	0.49	5 - 50
Zinc	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.70	3.39	5 - 30
Molybdenum	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.09	0.04	0.25- 2

\* On the basis of 20 T. manure containing 80 per cent moisture

\*\* Range of recommendations of various workers as found in the literature

together with an estimate of the range of soil applications per acre used by various workers as found in the literature, are presented in Table 3. With the exception of zinc, the maximum quantities of the elements in manure are somewhat less, and the average quantities considerably less, than the minimum amounts commonly used when treatment is applied as a chemical compound. Nevertheless, regular applications of manure should be sufficient in many cases to prevent or delay the appearance of disorders due to a deficiency of one or more of the trace elements.

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# CYTOGENETIC STUDIES OF STERILITY IN RYE<sup>2</sup>

E. D. PUTT<sup>2</sup>

*Experimental Station, Morden, Man.*

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## ABSTRACT

Pollen abortion and seed set in 11 long-term rye inbred lines was highly variable, significant differences occurring between progeny means and in some instances between progenies from the same grandparent plant. No consistent association was observed between the two characters. Cytological aberrations in the microsporocytes included univalents, sticky chromosomes, inversions, long chromosomes, stretched rod bivalents and a translocation, but in many plants they were insufficient to account for the high pollen abortion and low seed set. One line was uniform for long chromosomes.

In a cross of two inbreds, pollen abortion and low seed set appeared to be inherited but on a highly complex basis. In one family of this cross cytoplasmic control of pollen abortion was indicated. A translocation, tripolar spindles and six normal bivalents plus two bivalent fragments at diakinesis were observed; the latter two aberrations in sib progenies suggesting they were inherited.

Of nine progenies of the Emerald variety grown from open-pollinated low seed setting spikes, three showed a translocation and three others extra chromosome fragments.

Limited investigation suggested production of lines uniform for low pollen abortion and high seed set was possible.

Methyl alcohol was used successfully as a substitute for ethyl alcohol as a component for killing and preservation of the microsporocytes.

Rye, *Secale cereale* L. is grown as a cultivated cereal crop in many regions of the world. While Europe is credited with the largest quantity it is also of economic importance in certain regions of North America, including Western Canada and the states of North Dakota, South Dakota and Minnesota. It is generally recognized as being a valuable crop on sandy soils or soils of low fertility. Its greater hardiness than other cereals makes it useful as a winter cover crop for soils subject to erosion. One rather common defect of rye is the failure of a large portion of the florets to set seed under field conditions.

Preliminary observations in 1941 showed considerable pollen abortion in  $F_1$  plants from crosses between inbred lines in the breeding plots of the Division of Agronomy and Plant Genetics. The original plan for this study was to cross the inbred lines with a normal stock and study the  $F_1$  and later generations cytologically. As no line with normal pollen fertility could be found the inbred lines together with the progeny of one of the crosses between two of them were studied cytologically instead. Efforts were made also to develop a line uniform for low pollen abortion for future studies.

<sup>1</sup> Contribution from the Division of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota. Paper No. 2922, Scientific Journal Series, Minnesota Agricultural Experiment Station. Part of a thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup> Formerly Agronomist, Co-op. Vegetable Oils Ltd., Altona, Manitoba; now Agrostologist, Experimental Station, Morden, Manitoba.

## MATERIALS AND METHODS

The source of the selfed lines and the number of generations that each had been selfed at the start of the study in 1946 are in Table 1. The lines were in two groups, C.P. 1 to C.P. 8 having been selected for colourless seed, C.P. 9 to C.P. 15 for green seed. Diallel crosses within each group were growing in the breeding nursery of the Division of Agronomy and Plant Genetics in 1941 at which time preliminary observations were made by C. R. Burnham on the pollen abortion of the  $F_1$  plants. These results are given in Table 2. All lines except C.P. 1 and C.P. 8 produced pollen abortion in certain of the crosses or in part of the plants in  $F_1$  of the crosses studied. Two crosses were missing in one group and one in the other. The amount of pollen abortion varied greatly between the different lines. C.P. 2 was recorded as normal in all crosses except with C.P. 4, in which there was 25 per cent abortion observed in certain plants. At the other extreme was line C.P. 9 which showed high abortion in combinations with all the other inbreds in its group.

The other noticeable feature of these data was a lack of uniformity of the pollen abortion within several of the crosses. A number had plants with normal pollen and others with up to 50 per cent abortion. Particularly notable in this category were the crosses involving line C.P. 3. Also in the cross C.P. 9  $\times$  C.P. 15 where no normal plants were recorded there were plants with 30 per cent and others with 90 per cent pollen abortion.

The first active work on the study commenced in 1946. In that season, remnant 1939 seed of all the inbred lines, except C.P. 1 was planted to obtain fresh seed of the various lines. In addition a sample of bulk Dakold, selfed two generations and  $F_1$  seeds of a few of the original crosses

TABLE 1.—SOURCES OF THE SELFED LINES OF RYE AND THE NUMBER OF GENERATIONS EACH HAD BEEN SELFED AT THE START OF THE STUDY IN 1946

Number of line	Varietal source	Generations selfed
C.P. 1	Sagnitz	11
*2	Minn. 104	11
*3	Minn. 104	13
4	High $\times$ High	8
5	High $\times$ Low	9
*6	Minn. 111	5
7	Minn. 111	5
*8	Dakold	3
*9	German	9
*10	Minn. 104	13
*11	Minn. 2	12
12	High $\times$ Low	9
13	Minn. 113	5
*14	Minn. 113	5
*15	Dakold	3
P.C. *36	Minn. 104	12
*40	Minn. 104	12

\* Lines in which plants were produced in 1946 from 1939 remnant seed obtained through the courtesy of H. K. Hayes. Seed of the other lines, except C.P. 1 which was not available, failed to germinate.

TABLE 2.—ESTIMATED PER CENT POLLEN ABORTION IN  $F_1$  HYBRIDS OF SELFED LINES IN PLANT BREEDING NURSERY 1941. THE FEMALE PARENT IS LISTED HORIZONTALLY, MALE PARENT VERTICALLY. N = ALL PLANTS NORMAL; N, -25 = PLANTS WITH NORMAL POLLEN, OTHERS WITH 25 PER CENT ABORTION

C.P. No.	1	2	3	4	5	6	7
2	N						
3	N	N					
4	N	N, -25	N, -25				
5	N	N	N, -30	N, -50			
6	N	N	N, -25	50	50		
7	N	N	N, -50	N	—	N, -50	
8	N	N	N	N	—	N	N

C.P. No.	9	10	11	12	13	14
10	50					
11	50	N				
12	50	N	N			
13	50	N	—	N		
14	75, -90	N	Low	N	N	
15	30, -90	N	Low	N	N, -50	N

were included. Part of the material was grown at St. Paul in the greenhouse during the winter and spring and part at Truro, Nova Scotia, in outside flats after being started in a greenhouse.

Only 11 of the 17 lines had germinable seed and produced plants which were selfed. Selfed seed was obtained from  $F_1$  plants of some of the original crosses, in particular, the cross C.P. 9  $\times$  C.P. 14 which was given major consideration in the latter part of the study.

The second lot of material was started mainly from the 1946 selfed seed, in February of 1947 in the greenhouse at St. Paul. After a cold shock, the major portion was transplanted to the field. Besides the material already mentioned, progenies were grown from open pollinated seed from nine spikes of the Emerald variety, that had been selected for low seed setting. These were from an increase field grown at University Farm in 1946.

New crosses were planned in 1947 between the selfed lines and between them and any stock with normal pollen fertility. This plan was not carried out since pollen examination showed a considerable degree of sterility in all lines available including the selfed lines. The plan was shifted to a study of the breeding behaviour within the inbred lines and of one cross involving line C.P. 9, the line which in all  $F_1$  crosses grown in 1941 had



shown 50 per cent or more pollen abortion. To build up a line with normal pollen production or at least low pollen sterility and good seed setting ability, plants identified in 1947 and 1948 as having low pollen abortion were crossed reciprocally whenever possible and also selfed.

The material for study in 1948 and 1949 was started in the previous fall at Altona, Manitoba. It included the crosses made between plants of low pollen sterility during the previous summer and whenever available, selfed seed of the parents. Progenies were grown from all the inbred lines recovered in 1946, except C.P. 6, for which no selfed seed was obtained in 1947. Usually there was selfed seed available from only one or two plants of each of the selfed lines. Where a choice was available, plants of lowest pollen sterility were used to continue them. A progeny of the Dakold variety selfed for three generations was grown in 1948 from the remnant seed because the 1947 data had shown it to be a good source of plants with low pollen sterility. Emphasis was placed on material from the cross C.P. 9  $\times$  C.P. 14.

The Dakold progeny selfed three generations on which pollen sterility, seed set and quartet (tetrad) data were obtained was used for comparison with other material. It is termed "Dakold S<sub>3</sub>" in the remainder of this paper. A further group of 23 open-pollinated plants of the Dakold variety collected at University Farm, St. Paul, in 1948 and on which only pollen and quartet data were secured was also used for comparisons with the selfed lines. It is referred to as "U. Farm Dakold".

Pollen and mature head samples were collected from all plants possible in the three years 1947 to 1949 and sporocyte samples on approximately one-half of the plants in each progeny. The progenies varied from 2 to 23 plants depending on the amount of seed available and the success in establishing plants from it. Most progenies consisted of 10 to 12 plants.

In the collection of sporocyte samples one to three spikes were killed in freshly prepared solution, consisting of three parts of 95 per cent ethyl alcohol and one part of glacial acetic acid. The correct stage was found to vary from the time when the terminal leaf sheath was exposed about two inches, up to the early appearance of the awn tips. After 24 to 48 hours, the killer was replaced by two changes of 70 per cent alcohol. Subsequent storage was at room temperature.

Difficulty was met in securing ethyl alcohol. Because of this methyl alcohol was substituted in the 1949 sporocyte collections. It was also used instead of ethyl alcohol as the first change and on part of the samples for the second change. Figures of diakinesis and metaphase coming from samples preserved in this way were satisfactory. The results merit investigation of this cheaper and more easily obtainable alcohol for wider use in collection and preservation of sporocytes.

Sporocytes were examined with the aceto-carmin smear technique. A number of slides were made permanent by the method in use at the cytogenetics laboratory at the University of Minnesota. It is essentially McClintock's method with a few steps omitted. The cover slip was floated off in 10 per cent glacial acetic acid, then both it and the slide were passed through four solution changes in stender dishes as follows: (1) 20 cc. glacial acetic acid 20 cc. absolute ethyl alcohol (2) 4 cc. glacial acetic acid

36 cc. absolute ethyl alcohol (3) 40 cc. absolute ethyl alcohol (4) 40 cc. absolute ethyl alcohol. Minimum time in each solution was two minutes. They were made fresh each day. Following these solutions the slide was mounted in Canada Balsam.

Sporocytes chosen for examination, as a general rule, consisted of those from the parent plants of the progenies and, in the 1947 and 1949 material, the plant of lowest and highest sterility which had been collected in each progeny.

As one indication of cytological abnormality, the percentage of quartets showing micronuclei was determined on all sporocyte samples examined, except a few which were too young. These were made at 430X or 562X magnification, and whenever possible, based on at least 200 quartets from a single anther for each plant studied. They are shown as per cent abnormal quartets in the data which follow. In the 1947 and 1948 material, metaphase was also examined for gross abnormalities at this stage. When located during the search for the above stages, diakinesis, first division anaphase, and second division figures were also studied.

Pollen samples were collected by taking a whole spike at anthesis and storing it in 70 per cent alcohol or the decanted killer and first change alcohol from the sporocyte samples.

In pollen sterility examinations considerable difficulty was usually experienced due to the tendency of aborted grains to float to the edge of the drop of iodine solution. To avoid this, an iodine solution was prepared by dissolving 0.6 grams of iodine and two grams of potassium iodide in a mixture of 50 cc. of water and 50 cc. of glycerine. This type of solution was more viscous than an aqueous solution and to a considerable extent it corrected this source of error. The slides could also be kept three weeks to a month without drying out and without any serious destaining.

Considerable variability in the amount of visible abortion between different anthers of the same plant was observed. Following some study the technique adopted was to prepare a slide for each plant by teasing out the contents of three anthers of different florets into a large drop of the iodine solution and spreading it under a coverslip. These slides were compared with standards at class centres of 15.0 per cent abortion and upwards in 10 per cent gradations determined by actual count of 1500 to 2000 grains for each. Two centres of 2.5 per cent and 7.5 per cent were established for the plants of lower pollen abortion because selection for low sterility was an important consideration in this work. Those plants which failed to yield any normal pollen grains from the three anthers were classified as male sterile (M.S.). In some instances the male sterile condition appeared to be due to poor dehiscence, and what appeared to be normal grains were found in large clumps. In other instances, free grains were observed on the slide but none of them contained starch. In plants classified as male sterile the anthers were usually shorter than normal.

In determining seed setting the number of lateral florets of one to six open-pollinated spikes per plant was counted, exclusive of the lower and upper spikelets which often do not set seed, the seeds rubbed out, counted and the percentage of seed set for each plant determined. Usually the number of florets varied from 100 to 200. Any plants with less than 50

florets were not considered. In using this method, a few plants were found which had a seed setting percentage over 100. This anomaly was probably due to seeds in the lowermost spikelet or in occasional multiple florets.

In all analyses involving data expressed in percentages the values have been transformed to degrees of  $\sin^2\theta$ . Where means have been compared, they are the means of the transformed values rather than of the observed values. Both are reported in the tables. For purposes of calculation male sterile plants have been considered as having 100 per cent abortion and those few plants with seed set over 100 per cent as having only 100 per cent. The percentage of normal quartets has been used rather than the percentage of abnormal quartets as determined, in order to avoid zeros.

## EXPERIMENTAL RESULTS

### *Emerald Open-pollinated Progenies*

As a check on the possible cytological causes of low seed set in the field a search was made in 1946 by C. R. Burnham for such heads in an increase plot of Emerald rye under open-pollination. This variety was developed at University Farm, St. Paul, by selection and combination of self-pollinated lines. Seed set on a high proportion of the heads that year was nearly 100 per cent but nine heads with low seed set were collected. Progenies were grown from the open-pollinated seed of these spikes in 1947 as a part of the present study.

Cytological examination has shown that three of them contained plants with a ring or chain of four chromosomes at metaphase, demonstrating that the parent plants were heterozygous for a reciprocal translocation. It has not been determined if the same translocations were present in the three progenies. The ring was observed to be free of the nucleolus in each.

Plants in another three progenies showed the normal chromosome complement, with an added paired fragment or small chromosome at diakinesis (Figures 1 and 7). At metaphase in these plants two small univalents were frequently observed.

In the remaining three progenies no cytological aberration was noted.

These abnormalities may have been in one or more of the inbreds used in producing the Emerald variety; or they may have occurred during or since its origin.

### *Description of "U. Farm Dakold" and "Dakold S<sub>3</sub>"*

Throughout the study comparisons have been made with two groups of plants designated Dakold S<sub>3</sub> and U. Farm Dakold. The former was a Dakold progeny selfed three generations and consisted of 23 plants grown at Altona in 1948. The U. Farm Dakold sample comprised 23 open-pollinated plants grown at University Farm, St. Paul in 1948. Data on per cent pollen abortion, seed set and abnormal quartets (tetrads) for the Dakold S<sub>3</sub> and per cent pollen abortion and abnormal quartets for the U. Farm Dakold are presented in Table 3. The range in pollen abortion for each group was from 2.5 to 15.0 per cent with a mean of 6.7 for one group and 5.9 for the other. The range in seed set in the Dakold S<sub>3</sub> was from 42.8 to 100 per cent, a much greater range than for pollen abortion. The mean seed set value was 76.0 per cent. As a whole the two groups



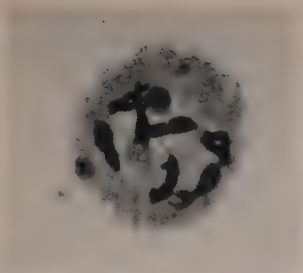


FIG. 1

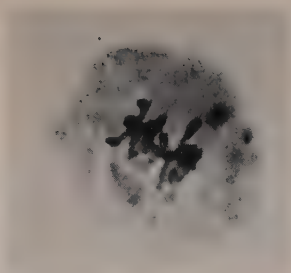


FIG. 2



FIG. 3

FIGURE 1. Diakinesis of normal Dakold showing seven bivalents.

FIGURE 2. Metaphase I of normal Dakold rye showing two rod bivalents and the remainder ring bivalents.

FIGURE 3. Long chromosome condition of line C.P. 11 at metaphase I.

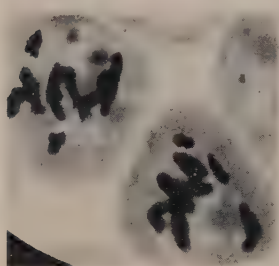


FIG. 4



FIG. 5

FIGURE 4. Diakinesis showing six bivalents and two paired fragments, found in Line C.P. 14 and  $F_3$  of the cross C.P. 9  $\times$  C.P. 14. Compare with Figure 1.

FIGURE 5. Two bridges and two fragments at anaphase I in line C.P. 14 indicating paracentric inversions.



FIG. 6

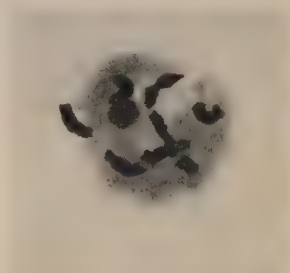


FIG. 7

FIGURE 6. Tripolar spindle condition in  $F_3$  of cross C.P. 9  $\times$  C.P. 14. Note cell at upper left appears normal illustrating the variable expression of this character.

FIGURE 7. Diakinesis showing seven bivalents and a short paired fragment found in three of nine Emerald progenies produced by open-pollination. Compare with Figures 1 and 4.

(Magnification Figure 6, 450X; all others, 970X.)





TABLE 3.—PER CENT POLLEN ABORTION, SEED SET, ABNORMAL AND NORMAL QUARTETS IN OPEN-POLLINATED DAKOLD RYE AND A DAKOLD LINE SELFED THREE GENERATIONS

	Dakold U. Farm (open-pollinated)			
	Number of plants	Observed range	Observed Means	Transformed
Pollen abortion	23	2.5- 15.0	6.7	14.1 $\pm$ 1.16
Abnormal quartets	23	0.0- 3.8	0.9	—
Normal quartets	23	96.2-100.0	99.1	85.6 $\pm$ 0.72
	Dakold S <sub>3</sub> (selfed three generations)			
	Number of plants	Observed range	Observed Means	Transformed
Pollen abortion	23	2.5- 15.0	5.9	13.3 $\pm$ 1.03
Seed set	23	42.8-101.0	76.0	62.0 $\pm$ 2.55
Abnormal quartets	17	0.0- 2.3	0.5	—
Normal quartets	17	97.7-100.0	99.5	87.1 $\pm$ 0.66

\* TABLE 4.—CORRELATION COEFFICIENTS FOR THE ASSOCIATIONS BETWEEN PER CENT POLLEN ABORTION, SEED SET AND NORMAL QUARTETS IN OPEN-POLLINATED DAKOLD RYE AND A DAKOLD LINE SELFED THREE GENERATIONS

Characters	Material	n	"r" value
Pollen abortion and seed set	Dakold S <sub>3</sub>	23	- 0.023
Normal quartets and seed set	Dakold S <sub>3</sub>	17	- 0.117
Normal quartets and pollen abortion	Dakold S <sub>3</sub>	17	- 0.175
Normal quartets and pollen abortion	U. Farm Dakold	23	- 0.121

had little cytological abnormality as shown by a mean of less than 1 per cent of abnormal quartets in both instances. However, certain plants in both groups had some abnormal cytological behaviour. The U. Farm group had one plant with 3.8 per cent abnormal quartets and the Dakold S<sub>3</sub> line a plant with 2.3 per cent. The correlation coefficients for the association of these three characters are shown in Table 4. Correlation between pollen abortion and seed set in the Dakold S<sub>3</sub> gave an "r" value of only - 0.023, much below the 5 per cent level of significance. The percentage of normal quartets also showed no significant correlation with either pollen abortion in both groups or seed set in the Dakold S<sub>3</sub>.

Plants of the Dakold S<sub>3</sub> and U. Farm Dakold had metaphase I figures containing zero to four rod bivalents, the average number being estimated at two, with the remainder in most cells appearing as ring bivalents (Figure 2). Anaphase I showed frequent short thick bridges suggesting delayed separation. Occasionally a long bridge was found suggesting an inversion but no associated fragment was noted. The plants also usually showed an

occasional univalent or, less frequently, a bivalent which was off the metaphase plate. The data are considered descriptive of the variation which may be found in open-pollinated and short-term inbred rye for the characters studied.

### *Behaviour of the Selfed Lines*

The sources of the selfed lines and the number of generations that each had been selfed at the start of the study are in Table 1. Detailed data will be presented on the two lines C.P. 10 and C.P. 11. The former was the line most extensively studied and shows behaviour considered typical of the selfed lines. C.P. 11 was of special interest in that it appeared uniform for an unusual chromosome condition. Lines C.P. 9 and C.P. 14 will also be discussed in detail because they were the parents of the one cross which was examined in most detail.

### *Line C.P. 9*

According to the 1941 observations this line in combination with all other lines in its group produced plants with high pollen abortion (Table 2). It had been selfed for nine generations at the time those crosses were made.

The seed of C.P. 9 for the 1940 crossing plots was recorded as having come from two 1939 plants row 3 plant 5 and row 3 plant 7. Spikes from five plants in the progeny tracing back to the latter, grown in 1941, had been preserved and were available for pollen examination. These showed a range in pollen abortion from 25.0 to 85.0 per cent with an observed mean of 51.0 per cent.

In 1946, seed from 1939 row 3 plant 4 was grown at Truro, Nova Scotia, and self seed produced. Two plants grown from this seed in 1948 were in the 95.0 per cent pollen abortion class. The one examined cytologically had only 5.3 per cent of abnormal quartets. The most noticeable deviation from normal behaviour was that all stages from metaphase to quartets were observed in a single anther. Another feature was the frequent occurrence at telophase I of one or two chromosome fragments attached to the main chromatin body by thin threads. The meiotic irregularities were seemingly insufficient to account for the high pollen abortion. Efforts to secure self seed from these two plants were not successful, thus eliminating this branch of the line.

A second branch of the line C.P. 9 was established in 1946 from self seed of a plant grown in the 1940 crossing plot. It was open-pollinated in the greenhouse in 1946. The bulk of the material growing at the same time consisted of the selfed lines listed in Table 1, a number of the original diallel crosses and a two-year selfed Dakold culture, so that the pollen for the open-pollination may have constituted a highly heterogeneous sample. A single progeny following the open-pollination was grown in 1947. The one plant that flowered had only 2.5 per cent of pollen abortion and normal cytological behaviour. This plant was selfed, its progeny grown in 1948, and a two year self progeny in 1949. A summary of the per cent pollen abortion and seed set is given in Table 5 for these progenies. Though the pollen abortion was higher in the C.P. 9 progenies than in the Dakold  $S_3$  and the U. Farm Dakold, only the difference between the 1948 progeny and the Dakold  $S_3$  was significant at the five per cent level. The 1949 progeny



TABLE 5.—PER CENT POLLEN ABORTION AND SEED SET IN TWO PROGENIES OF LINE C.P. 9, SELF-POLLINATED NINE GENERATIONS, OPEN-POLLINATED ONE GENERATION, THEN SELFED ONE AND TWO GENERATIONS, RESPECTIVELY, 1948 and 1949

Number	Pollen abortion			Means	
	Year	Number of plants	Observed range	Observed	Transformed
35	1948	12	2.5–15.0	8.3	16.5 ± 1.02
25	1949	10	2.5–85.0	22.5	25.6 ± 6.18
Dakold S <sub>3</sub>	1948	23	2.5–15.0	5.9	13.3 ± 1.03
U. Farm Dakold	—	23	2.5–15.0	6.7	14.1 ± 1.16
Seed set					
35	1948	8	11.1–83.0	55.1	47.8 ± 4.86
25	1949	10	0.0*–86.0	46.6	41.6 ± 6.06
Dakold S <sub>3</sub>	1948	23	42.8–101.0	76.0	62.0 ± 2.55

\* Considered as 0.1 per cent in calculating transformed mean.

showed a wide range of abortion in the pollen. The mean seed set was significantly lower than the Dakold S<sub>3</sub>. There was also a wide range in seed set in both progenies. Two plants of the 1949 progeny showed only 2.0 and 0.5 per cent of the quartets with micronuclei, indicating normal or nearly normal meiosis.

The data for line C.P. 9 suggest that it was heterozygous or still segregating for factors affecting pollen sterility. The plants grown in 1941, as well as the two in 1948 descending through continuous selfing, indicate that a consistently high degree of pollen sterility existed in the line. The data on the F<sub>1</sub> plants grown in 1941 show that this sterility was inherited through the female side in all crosses with other inbred lines. The data from the branch of the line passing through one generation of open-pollination were entirely different. The one plant flowering in the 1947 progeny from open-pollination had only 2.5 per cent pollen sterility. Presumably this plant would be the result of cross fertilization and would be an F<sub>1</sub>, yet it did not show the high sterility characteristic of the F<sub>1</sub>'s resulting from controlled crossing. The genes causing high sterility could have been recessive to the genes carried by the particular pollen grain which produced the gamete entering into the zygote. But the F<sub>2</sub> progeny from selfing this plant did not produce any plants with high pollen sterility. A few plants of high sterility did reappear in the 1949 or F<sub>3</sub> progeny. Thus it appears that the C.P. 9 line must have lacked uniformity for pollen sterility, that it had at least two types of plants, those with the ability to cause sterility when outcrossed and others which lacked it.

#### Line C.P. 10

A single plant of this line was selfed in 1946. In each of the years 1948 and 1949 four progenies were grown which descended from this plant, these having been selfed 15 and 16 generations respectively. A summary of the pollen abortion and per cent seed set for these progenies is given in Table 6.

These data show that all progenies had a higher mean pollen abortion than either the U. Farm Dakold which was open-pollinated or the Dakold S<sub>3</sub> progeny, certain of the differences being significant. The differences

between progenies 36 and 53 grown in 1948 approached significance and those between 1949 progenies 34 and 35 and 35 and 37 exceeded the five per cent level. At this level only one significant difference would be expected out of 20 comparisons due to chance alone. The existence of two significant differences among the six comparisons in the 1949 progenies is an indication that factors other than chance were operating, and indicates segregation for factors affecting pollen abortion. There were also significant differences between the progenies grown in the different years, but these may not be valid comparisons since no information on the environmental effect on pollen abortion is available.

TABLE 6.—PER CENT POLLEN ABORTION AND SEED SET IN THE PROGENIES OF SELFED LINE C.P. 10 (1948 PROGENIES SELFED 15 GENERATIONS, 1949, 16 GENERATIONS)

Number	Pollen abortion			Means	
	Year	Number of plants	Observed range	Observed	Transformed
29	1948	12	2.5– 55.0	10.2	16.3 $\pm$ 3.25
36	1948	13	2.5– 45.0	14.8	21.1 $\pm$ 2.75*
52	1948	10	2.5– 75.0	21.5	21.8 $\pm$ 4.24
53	1948	22	2.5– 45.0	8.1	15.2 $\pm$ 1.57
34	1949	15	2.5– M.S.	34.7	36.6 $\pm$ 7.09**
35	1949	10	2.5– 25.0	9.8	17.3 $\pm$ 2.02
36	1949	16	2.5– 95.0	18.4	24.1 $\pm$ 3.90*
37	1949	13	2.5– 55.0	29.0	31.3 $\pm$ 3.48**
Dakold S <sub>3</sub>	1948	23	2.5– 15.0	5.9	13.3 $\pm$ 1.03
U. Farm Dakold	—	23	2.5– 15.0	6.7	14.1 $\pm$ 1.16
Seed set					
29	1948	7	38.7– 96.4	77.5	64.4 $\pm$ 6.51
36	1948	8	49.1– 89.3	71.0	58.2 $\pm$ 2.75
52	1948	5	27.6– 86.7	67.3	55.8 $\pm$ 6.44
53	1948	12	37.4– 88.6	69.2	56.8 $\pm$ 2.66
34	1949	15	49.3– 83.3	67.4	55.5 $\pm$ 1.65
35	1949	9	32.9– 69.8	59.3	50.5 $\pm$ 2.70
36	1949	16	36.1– 83.5	59.9	50.9 $\pm$ 1.81
37	1949	13	47.6– 80.8	63.5	53.0 $\pm$ 1.50
Dakold S <sub>3</sub>	1948	23	42.8–101.0	76.0	62.0 $\pm$ 2.55

\* Higher than Dakold S<sub>3</sub> at 5 per cent level.

\*\* Higher than Dakold S<sub>3</sub> at 1 per cent level.

TABLE 7.—CORRELATION COEFFICIENTS FOR THE ASSOCIATION OF PER CENT POLLEN STERILITY WITH PER CENT SEED SET IN PROGENIES OF SELFED LINE C.P. 10

Number	Year	n	"r" value	"r" value required for significance at the 5 per cent level
29	1948	7	- 0.094	0.754
36	1948	8	- 0.745*	0.707
52	1948	5	- 0.866	0.878
53	1948	9	+ 0.196	0.666
34	1949	15	+ 0.305	0.514
35	1949	9	- 0.575	0.666
36	1949	16	- 0.469	0.497
37	1949	13	- 0.090	0.553
Mean by "Z" transformations			- 0.221	0.250

The data on per cent seed set, Table 6, show that all progenies except one had a lower mean value than the Dakold S<sub>3</sub> grown in 1948, but none of the differences was significant for the 1948 progenies. Seed set data in 1949 for Dakold S<sub>3</sub> were not available. Between progenies grown in the same year only the 1949—34 and 36 difference approached significance. This and the wide range of seed set for all progenies shown in Table 6 suggest that the parent plant may have been heterozygous for factors affecting seed setting as well as for pollen abortion.

Correlation coefficients for the association of pollen sterility with seed set are in Table 7. Six of the eight were negative but only one exceeded and another approached the level required for significance at the five per cent point. Averaging the individual values by means of "Z" transformations gave a value of only -0.221, still below the five per cent level of 0.25. Evidently the factors which caused abortion of the pollen did not have any consistent relation with seed setting.

This is supported also by a study of the association of per cent normal quartets with seed set and with pollen abortion. The correlation coefficient for the association with pollen abortion for 20 paired values from individual plants was -0.488, a value which exceeded the five per cent level. The value for the association with seed set for 18 pairs was only +0.56. These coefficients indicated that cytological aberrations as measured by abnormal quartets were contributing to pollen abortion in a fair degree, but not to seed set.

A summary of the cytological behaviour of selected plants of line C.P. 10 is given in Table 8. The existence of univalents and the occurrence of several stages in one anther were the most commonly observed abnormalities. In many instances, the abnormalities were not consistent with the observed pollen abortion and seed set. Plants 48-53-1 and 48-53-2 had

TABLE 8.—MEIOTIC BEHAVIOUR AND PER CENT POLLEN ABORTION, SEED SET AND ABNORMAL QUARTETS OF PLANTS OF SELFED LINE C.P. 10

Year, progeny and plant	Per cent pollen abortion	Per cent seed set	Per cent abnormal quartets	Meiotic behaviour
47-58-2	2.5	—	0.3	Univalents in one to two per cent of cells, excess of rod bivalents, metaphase I to interphase in same anther.
47-58-7	2.5	—	1.3	Normal, except for rare univalent.
47-58-8	7.5	—	—	Normal, except for rare univalent.
48-52-2	75.0	27.6	—	Univalents, up to six, in 40 per cent of cells, ring or chain of four chromosomes in some cells, others with seven bivalents. High frequency of rod bivalents. Several stages in one anther.
48-52-4	2.5	81.9	2.3	Univalents, up to four, in 15 per cent of cells. Several stages in one anther.
48-53-1	7.5	60.9	8.0	Two long rod bivalents frequent, univalents in one to two per cent of cells.
48-53-2	7.5	59.9	2.8	Normal
48-53-3	45.0	75.9	8.4	Univalents in 10 per cent of cells, excess rod bivalents, frequent lagging at anaphase II.
48-53-4	15.0	78.7	1.9	Normal except for excess rod bivalents.
48-29-1	15.0	83.2	2.6	Diakinesis to quartets in same anther.
48-29-6	2.5	90.2	3.4	Normal.
48-36-7	2.5	89.3	0.4	Normal.
48-36-8	45.0	49.1	3.7	Univalents in 10 per cent of cells, three to four stages in one anther.

widely different percentages of abnormal quartets but about the same seed set and the same pollen abortion. Plants 48-53-3 and 48-36-8 had the same pollen sterility and greatly different seed set, yet there was only a small difference in the frequency of abnormal quartets which was the reverse of the per cent seed set. Such information indicates that other factors in addition to the observed cytological abnormalities were affecting pollen abortion and seed set.

One plant in which part of the male and female abortion could be explained by the cytological aberration is 48-52-2. This plant had numerous univalents and a ring or chain of four chromosomes showing that it was



heterozygous for a reciprocal translocation. Even in this plant, however, the per cent pollen abortion was higher and the per cent seed set lower than expected as a result of the translocation especially since it formed a configuration of four only part of the time, and may have caused less than 50 per cent abortion. The high frequency of univalents was probably also contributing to reduced seed set and high pollen abortion.

There was no known source of pollen contamination which could have caused this translocation to appear. It may have existed in the original plant from which the inbred arose, but the probability of its being continued through 15 generations of selfing is extremely low; especially since plants with low seed set were avoided in continuing the inbred lines. A more probable explanation is that a spontaneous translocation occurred in the parent of plant 48-52-2 especially since at least one instance of this has been reported (14) in rye and there is considerable evidence of meiotic variability in this line.

Another observation which indicates line C.P. 10 was heterozygous was the occurrence of albino plants. In progeny 52 there were 21 normal and 12 albino seedlings. The progeny of 48-52-4 included 40 normal and 10 albino seedlings. Chi-square tests on these data show satisfactory fits to a 3 : 1 ratio. How long this albino had been carried in the line is not known.

#### *Line C.P. 11*

A single plant of this line in an 11th generation self progeny flowered in 1947. It was estimated to have 45.0 per cent pollen abortion. It was self-pollinated and used as the male parent in crosses with line C.P. 10. The progeny from selfing, grown in the field in 1948, had uniformly high pollen abortion and plants of it were crossed reciprocally with  $F_1$  plants in two progenies which were crosses between plants in two and three year selfed Dakold. These two cross progenies from the two and three year selfed Dakold had uniformly low pollen abortion and high seed set and had come from a reciprocal cross of two plants with low pollen sterility. The  $F_1$  plants from the crosses with C.P. 11 and progenies from selfing the two C.P. 11 parent plants were grown at Altona in 1949.

A summary of the data for pollen abortion and seed set for the 1948 and 1949 material is given in Table 9. Tests on the means of these data showed that the pollen sterility of the C.P. 11 progenies was higher than the U. Farm Dakold, the Dakold  $S_3$  progeny, and the  $F_1$ 's, and by an amount greater than that required for significance at the one per cent level. The pollen abortion of the  $F_1$  plants was of the same order as the U. Farm Dakold and the Dakold  $S_3$  in all instances, except 1948—13, which exceeded the Dakold  $S_3$  by an amount significant at the five per cent level.

In seed set the difference between the Dakold  $S_3$  and the two 1949 C.P. 11 progenies was significant at the five per cent level in one instance, and at the one per cent in the other. There was also a significant difference in seed set between the two 1949 progenies 28 and 29. The two  $F_1$  progenies did not differ significantly but both were higher than the Dakold  $S_3$ , the difference in one instance being significant at the one per cent level. Correlation coefficients for per cent pollen abortion and seed set were  $-0.089$ ,  $-0.181$  and  $-0.718$  for the three progenies 42, 28 and 29, respectively. The average value obtained by "Z" transformations was  $-0.249$ . None

TABLE 9.—PER CENT POLLEN ABORTION AND SEED SET IN THE PROGENIES OF SELFED LINE C.P. 11 AND IN F<sub>1</sub> HYBRIDS OF C.P. 11

Number	Pollen abortion				Means	
	Year	Description	Number of plants	Observed range	Observed	Transformed
42	1948	C.P. 11	12	55.0-95.0	73.3	60.0 ± 2.41
28	1949	C.P. 11	12	45.0-M.S.	80.0	57.8 ± 5.06
29	1949	C.P. 11	5	45.0-75.0	61.0	51.5 ± 3.03
13	1948	C.P. 10 × C.P. 11	7	7.5-15.0	10.7	17.9 ± 1.62
15	1948	C.P. 10 × C.P. 11	6	2.5-15.0	7.1	13.6 ± 2.28
17	1949	C.P. 11 × low sterile	6	2.5-7.5	4.2	11.4 ± 2.40
18	1949	Low sterile × C.P. 11	7	2.5-15.0	8.2	15.9 ± 2.11
Dakold S <sub>3</sub>	1948	—	23	2.5-15.0	5.9	13.3 ± 1.03
U. Farm Dakold	—	—	23	2.5-15.0	6.7	14.1 ± 1.16
Seed set						
42	1948	C.P. 11	6	50.5-98.0	67.0	56.4 ± 5.40
28	1949	C.P. 11	12	42.2-71.9	59.4	50.5 ± 1.58
29	1949	C.P. 11	7	0.0*-74.0	31.8	30.8 ± 8.30
17	1949	C.P. 11 × low sterile	6	80.8-100.0	89.5	73.0 ± 3.79
18	1949	Low sterile × C.P. 11	7	94.0-100.0	97.4	82.3 ± 2.99
Dakold S <sub>3</sub>	1948	—	23	42.8-101.0	76.0	62.0 ± 2.55

\* Considered as 0.1 per cent in calculating transformed mean.

of these values was significant, suggesting that the factors affecting pollen abortion and seed set were independent although the number of plants in the progenies was probably too small to constitute an adequate test.

The data of Table 9 show that the C.P. 11 line possessed an inherited condition which caused high pollen sterility in all plants. There was considerable range but all plants had pollen abortion higher than in the U. Farm Dakold and the Dakold S<sub>3</sub>. This condition seemed to be manifest in seed set also but to a lesser degree, the correlations suggesting no association between pollen abortion and seed set. There was also evidence that the condition was not genetically stabilized at a fixed level as shown by the significant differences which existed between the 1949 C.P. 11 progenies in seed set. The behaviour of the F<sub>1</sub> progenies resulting from crosses with plants of low pollen abortion and high seed set showed that the condition was recessive.

Metaphase I of meiosis was examined in six of the C.P. 11 plants grown in 1948 and one grown in 1949. In all instances the chromosomes were much longer than normal as though they had failed to contract normally (Figure 3). Rod bivalents and univalents were very frequent. Quartets examined in two of the 1948 plants and four of 1949 gave a range in per cent abnormality from 6.8 to 22.1 with an observed mean of 14.3. Correlation between per cent normal quartets and per cent pollen abortion for the six plants studied was -0.806, a high value, not significant but close to the five per cent level. With per cent seed set it was -0.462, opposite in sign to that expected and not significant, indicating that factors in addition to the observed cytological abnormalities may have been affecting pollen abortion and seed set. The numbers are small for sound conclusions.

Cytological examination of plants from the four  $F_1$  progenies showed normal metaphase and absence or a low percentage of abnormal quartets in all instances. This is further evidence of the condition in C.P. 11 being recessive.

Several of the  $F_1$  plants were selfed in 1949 and  $F_2$  progenies from six of them were grown in 1950. Progenies ranged from 16 to 27 plants. Pollen examinations were made on a total of 132 plants in these six progenies. Of the 132 plants only four came in classes with 45.0 per cent pollen abortion or greater, or possessed abortion equal to that found in the C.P. 11 parent. A very large majority, 103 plants, had pollen abortion in the 2.5 or 7.5 per cent class. There was evidence of differences between the progenies. Two possessed no plants with pollen abortion above the 15 per cent class and one other showed none above the 25 per cent class. Of the four  $F_2$  plants with 45 per cent or greater pollen abortion the highest three all occurred in a single progeny.

Examination of meiotic stages in a few plants of each progeny did not reveal any plants which could positively be classified as having an abnormally long chromosome condition similar to the C.P. 11 line. In three progenies no plants were discovered with any suspected abnormality in meiosis. In three other progenies plants were found with chromosomes longer than normal but observation did not suggest that their abnormalities were of the same degree as in the line C.P. 11. It was of further interest to note that the plants with the higher pollen abortion did not show the greatest frequencies of abnormalities. In fact the two plants with the greatest variation from normal or what could be considered as most similar to C.P. 11 in meiotic appearance had pollen abortion in the 25 and 35 per cent classes.

The results of the limited study of the  $F_2$  material suggest, due to the variation between the progenies, a lack of homozygosity in the parent material. It has already been observed that significant differences occurred within the C.P. 11 line and it is probable that the other parent was also heterozygous since it was not the product of selfing. The results also suggest that the inheritance of the long chromosome condition may be complex or at least that the condition, though apparently recessive is governed by more than a single major factor; and that it is not closely related to pollen abortion.

#### *Line C.P. 14*

This line together with line C.P. 9 which has already been discussed are of special interest since they were the parents of one cross which will be considered in detail in the latter part of this paper. Five progenies of line C.P. 14 selfed seven generations, and two selfed eight generations were grown at Altona in 1948 and 1949, respectively. The pollen abortion and seed set for these progenies are shown in Table 10. A seed set value (45.4 per cent) was secured on only one plant of 1948—28, and consequently this progeny is not listed.

Application of the "t" test to the differences between means for pollen abortion in Table 10 showed that significant differences at the one per cent level existed between the Dakold  $S_3$  and the U. Farm Dakold in comparison with all others and at the five per cent level between progenies 1948—72



TABLE 10.—PER CENT POLLEN ABORTION AND SEED SET OF PARENT PLANTS AND THEIR PROGENIES IN SELFED LINE C.P. 14 (1948 PROGENIES SELFED 7 GENERATIONS, 1949, 8 GENERATIONS)

Number	Pollen abortion				Means	
	Year	Number of plants	Parent plant	Observed range	Observed	Transformed
28	1948	4	7.5	15.0-65.0	35.0	35.7 ± 6.60
54	1948	6	25.0	45.0-75.0	63.3	52.9 ± 2.85
55	1948	11	7.5	15.0-55.0	34.1	35.3 ± 2.71
72	1948	8	25.0	15.0-65.0	40.0	38.7 ± 4.06
73	1948	7	7.5	15.0-35.0	23.6	28.7 ± 2.31
26	1949	11	15.0	7.5-35.0	22.0	27.1 ± 5.28
27	1949	4	15.0	7.5-25.0	15.6	22.9 ± 2.88
Dakold S <sub>3</sub>	1948	23	—	2.5-15.0	5.9	13.3 ± 1.03
U. Farm Dakold	—	23	—	2.5-15.0	6.7	14.1 ± 1.16
Seed set						
54	1948	2	—	17.8-27.7	22.8	—
55	1948	7	—	13.9-83.1	61.4	51.7 ± 5.76
72	1948	4	—	30.0-67.2	52.3	46.3 ± 4.67
73	1948	6	—	32.7-79.3	59.2	50.5 ± 3.94
26	1949	10	65.9	55.8-81.3	69.0	56.5 ± 1.91
27	1949	5	79.3	45.0-70.2	58.9	50.2 ± 2.57
Dakold S <sub>3</sub>	1948	23	—	42.8-101.0	76.0	62.0 ± 2.55

and 73 and 1948—28 and 54. Since the latter two comparisons were significant it is obvious that several other significant differences, some at the one per cent level, also existed in the 1948 progenies. These differences and the high range of sterility exhibited within several of the progenies showed that the C.P. 14 line was still heterozygous for factors controlling pollen abortion.

There is some evidence that the degree of pollen sterility of the parents was reflected in the progenies. Of five 1948 progenies the three from parents with 7.5 per cent abortion had a lower mean sterility than the two from parents with 25.0 per cent abortion. One progeny, 73, from a parent with 7.5 per cent pollen abortion had all its plants in the 35.0 per cent class or lower. In contrast, progeny 72, from a 25.0 per cent sterile parent, had four out of eight of its plants in the 45.0 per cent class or higher; and progeny 54, also from a 25.0 per cent sterile parent plant, had all of its plants in the 45.0 per cent class or higher. Also the two 1949 progenies from parents with 15.0 per cent abortion had the major portion of their plants in the lower sterility classes.

In seed set the mean differences between the Dakold S<sub>3</sub> and progenies 1948—27 and 1948—72 were significant at the one per cent level and for 1948—73 at the five per cent level. No significant differences existed between the C.P. 14 progenies themselves but the difference for 26 and 27 did approach the five per cent level. The mean for progeny 54 was lower than all the others but it was based on only two plants and cannot be seriously considered. These results suggest that the factors causing pollen abortion in this line did not have an equivalent effect in reducing seed set. The wide range in per cent seed set, however, does support the conclusion



TABLE 11.—MEIOTIC BEHAVIOUR AND PER CENT POLLEN ABORTION, SEED SET AND ABNORMAL QUARTETS OF PLANTS OF SELFED LINE C.P. 14

Year, progeny and plant	Per cent pollen abortion	Per cent seed set	Per cent abnormal quartets	Meiotic behaviour
47-60-1	15.0	—	14.6	Frequent, excessively stretched rod bivalent, occasional diagonal bridge in quartets.
48-54-2	65.0	27.7	21.2	Poor orientation at metaphase I, univalents in five to ten per cent of cells, bridge and fragment in few cells at anaphase I, small lagging fragments at anaphase II.
48-55-1	55.0	42.5	13.6	Univalents in five per cent of cells, few cells with bivalents slow to move to metaphase I plate, few cells with stretched rod bivalent.
48-72-3	45.0	30.0	5.3	Univalents in one per cent of cells, lagging fragment or bridge and fragment at anaphase in one per cent of cells. Jumbled metaphase I and abnormal stretching of chromosomes in one anther.
48-73-1	15.0	65.9	—	Univalents in one to two per cent of cells, diakinesis with six normal bivalents and two bivalent fragments.
48-73-2	25.0	68.0	7.5	Some diakinesis figures with six bivalents and two univalents, others with six normal bivalents and two bivalent fragments. Stretched rod bivalents at metaphase I, bridges and fragments in few cells at anaphase I.
48-73-3	35.0	50.6	8.3	Seven normal bivalents or six normal bivalents and two bivalent fragments at diakinesis, metaphase I normal, bridges and fragments at anaphase I, two cells noted with two bridges and two fragments.
49-26-8	35.0	55.8	3.0	Frequent evidence of six normal bivalents and two bivalent fragments at diakinesis, frequent stretched rod bivalent at metaphase I.

TABLE 12.—SUMMARY OF DATA SECURED FROM PROGENIES OF SELFED LINES

Line	Variation in per cent pollen abortion			Variation in per cent seed set				Correlations			Cytological observations
	Range within progenies		Between progenies	Range within progenies		Between progenies	Per cent pollen abortion vs. per cent seed set	Per cent normal quartets vs.			
	Maximum	Minimum		Maximum	Minimum			Per cent pollen abortion	Per cent seed set		
C.P. 2	48.5	18.5	Below*	53.3	15.4	*	- 0.469 to + 0.485	- 0.824**	+ 0.131	Inversion present Long chromosomes Excess univalents bridges at division II Excess univalents, translocation in one plant Uniform for long chromosomes Stretched rod bivalent, 6 bivalents plus 2 paired fragments at diakinesis and inversions present Similar to C.P. 14 but no inversions Long chromosomes Sticky chromosomes Five ring bivalents, two rod bivalents, rare univalent	
C.P. 3	85.0	***	—	71.8	—	—	- 0.968**	+ 0.245	—		
C.P. 8	48.5	***	—	52.4	—	—	- 0.768	—	—		
C.P. 10	98.5	22.5	*	59.1	32.2	*	- 0.221	- 0.488*	- 0.056		
C.P. 11	55.0	30.0	*	74.0	29.7	*	- 0.089 to - 0.718	- 0.808	- 0.462		
C.P. 14	88.5	18.5	**	70.0	30.0	Near*	- 0.568**	- 0.151	- 0.427		
C.P. 15	28.5	***	—	34.8	—	—	—	—	—		
P.C. 36	38.5	5.0	**	57.9	32.1	Below*	Less than 0.100	- 0.609	- 0.370		
P.C. 40	28.5	***	—	49.5	—	—	- 0.151	—	—		
Dakold	12.5	12.5	—	58.2	—	—	- 0.023 and	- 0.121	- 0.023		
								- 0.175			

\* Read as "five per cent level of significance" or "exceeds five per cent level of significance."

\*\* Read as "exceeds one per cent level of significance."

\*\*\* Only a single progeny grown.

reached from the pollen sterility data that line C.P. 14 was still highly variable and probably segregating. Comparison of progeny means for seed set with the means of the parents is possible only for the two 1949 progenies. For the two parents the difference was 13.4 per cent while the means of the two progenies showed a difference of 10.1 per cent but in the reverse direction.

Correlation coefficients for the association of pollen sterility and seed set were negative in four out of five progenies studied. None was significant. The one positive value was very high (0.950) but insignificant for only two degrees of freedom. Averaging the four negative values by means of "Z" transformations gave a value of  $-0.586$  for 13 degrees of freedom. This value exceeded the one per cent level indicating some association between pollen abortion and seed set in these four progenies as a group.

Quartet examination was made on 13 plants in this line. The range of abnormal quartets was from 3.0 to 21.2 per cent with an observed mean of 10.1 per cent. Correlation of the per cent of normal quartets with pollen sterility and seed set gave "r" values of  $-0.151$  and  $+0.427$ , both of which were non-significant indicating no definite relationship of cytological abnormality with microspore abortion or seed set.

A summary of the cytological behaviour of plants of the C.P. 14 line is given in Table 11. Only one 1947 plant is listed, but three sibs of this plant, in the same progeny of a single plant, showed similar behaviour. The 1947 plants were the parents of the other plants described. Two noticeable abnormalities occurred in this line. The most frequent was an excessively stretched rod bivalent. In many instances the stretching was so severe that the parts were attached by very slender chromatin threads visible only under 1125X magnification.

In several instances metaphase I plants occurred in a clumped condition with a portion of a chromosome attached to the clump by two slender threads. This was especially noticeable in plant 48-73-2 (Table 11). Also in this plant one cell showed a small fragment of a chromosome at one side of the metaphase plate and not visibly attached to the other chromosomes. It was not determined if such instances were another phase of the chromosome appearing as a stretched rod bivalent.

The other unusual phenomenon was the occurrence in plants of progeny 73 of six bivalents and two univalents or six bivalents and two short paired fragments (Figure 4). These apparently aberrant chromosomes were both much shorter than the normal pairs so that they were most probably the result of rearrangement within the normal chromatin complement rather than the extra chromosomes reported in the literature. It may be that similar behaviour was occurring in other progenies since diakinesis was only obtained in progenies 73 and 26. Plant 48-73-1 was the parent of progeny 49-26-8 in which the behaviour reappeared, showing that it was transmitted. Plant 49-26-8 also showed the stretched rod bivalent, this being the third successive generation in which this condition appeared. The abnormal diakinesis behaviour and the stretched rod bivalent may be manifestations of the same condition.

Line C.P. 14 also showed evidence of heterozygous inversions. Cells with the anaphase bridge and associated fragment characteristic of a hetero-

zygous paracentric inversion were found in three plants from three different progenies. One of these, 48-73-3 had at least two cells with two well separated bridges and two fragments, showing that at least two paracentric inversions were present (Figure 5).

### *Summary of Data for the Selfed Lines*

The salient data on the selfed lines for which one or more selfed progenies were studied, including six lines not already discussed are brought together in Table 12. The lines showed higher pollen abortion than the Dakold S<sub>3</sub> and the U. Farm Dakold for 30 out of 31 selfed progenies studied and lower seed set for 27 out of 28 progenies. In many instances these differences were significant.

In comparison with the Dakold S<sub>3</sub> and the U. Farm Dakold the inbred lines exhibited a much wider range of pollen abortion and this even though much smaller progenies were grown than for the Dakold samples. Only one progeny, out of P.C. 36, showed a lower range of pollen abortion than the Dakold S<sub>3</sub> and U. Farm Dakold. The data in Table 12 show that the inbred lines in this study were highly variable and that many were still segregating for factors controlling pollen abortion and to a lesser degree for those controlling seed set. In four out of five inbreds in which two or more progenies were studied significant differences between the mean pollen sterility of the progenies occurred. In some instances these differences existed for progenies from plants having the same parent. Such differences show that the individual plants within the inbreds were heterozygous at the start of the study. This heterozygosity is rather unexpected especially in inbreds C.P. 10 and 11 and P.C. 36 which had been selfed for 12 or more generations. The high variability within the single progeny studied in other inbreds suggests that they were also heterozygous for the factors affecting pollen sterility.

In seed set, the variability was also high but not as striking as for pollen sterility. Four out of nine inbreds listed in Table 12 showed at least one progeny with greater range in seed set than the Dakold S<sub>3</sub>, but the progeny of minimum variation in lines where more than one progeny was studied was less than for the Dakold sample in all instances. Three of the five inbreds in which two or more progenies were studied showed significant differences in the mean seed set between progenies and differences approaching significance in a fourth. These results indicate that the inbreds as a group were also heterozygous for factors affecting seed set.

Albino seedlings were segregating in lines C.P. 8, C.P. 10 and C.P. 15. This heterozygosity is unexpected also. They may represent new mutations but no information was available as to when they first appeared during the inbreeding.

As a general rule the inbreds showed no significant correlations for pollen sterility and seed set indicating different factors controlling pollen abortion and seed set in this material. The value for C.P. 3 did exceed the one per cent point but it was based on only a single small progeny, and cannot be considered seriously. The value of  $-0.568$  for C.P. 14, an average for four progenies, exceeded the one per cent point and may indicate



that some common factors were causing reduced seed set and pollen abortion in this line. This is somewhat doubtful, however, since another progeny gave a positive though insignificant value of 0.956.

Correlation of the per cent of normal microspore quartets as a measure of cytological aberration with seed set and pollen abortion failed to show any close relationship. Of those with pollen abortion, the values for C.P. 2 and C.P. 10 were significant and negative, but the others were not significant and were inconsistent in sign. Of those with seed set, none of the values was significant and the sign was also inconsistent. Other factors in addition to the cytological behaviour studied must have been affecting pollen abortion and seed set.

A number of cytological abnormalities have been noted which constitute further evidence of heterozygosity and variability in the inbreds. Heterozygous inversions were found in C.P. 2 and C.P. 14 and a heterozygous translocation in C.P. 10. Chromosome longer than normal were found in three lines, C.P. 3, C.P. 11 and P.C. 36, but their expression was variable in two out of the three. Only in C.P. 11 did the long chromosome character show in all plants examined. In fact this was the only line in which the cytological behaviour was reasonably uniform for all plants studied.

The study of these inbred lines has shown that they were exceedingly variable for pollen abortion, seed set and cytological behaviour, that the factors affecting pollen abortion and seed set were largely independent and that neither was consistently related to cytological aberration. Because they originated from six different sources it may be expected that this behaviour is a characteristic at least of long-time selfed lines in rye.

#### *Behaviour of Cross C.P. 9 × C.P. 14*

This cross was chosen for study because it involved line C.P. 9 which according to the estimates made by Burnham produced high pollen abortion when crossed with inbreds C.P. 10, 11, 12, 13, 14 and 15 (Table 2). This pollen sterility was uniformly 50 per cent for the  $F_1$  plants of all crosses except those from C.P. 14 and 15. Some of the plants of the C.P. 9 × C.P. 14 cross had 75 per cent pollen abortion and others 90 per cent. In the C.P. 9 × C.P. 15 cross some plants had 30 per cent pollen abortion and others 90 per cent.

The cross of C.P. 9 × C.P. 14 has been studied in five families descending from individual  $F_1$  plants. For the first three families the  $F_1$  plants were grown in the greenhouse in 1946. The other two were in the 1941 field plantings at St. Paul. In families 1 and 2 the  $F_1$  plants were self-pollinated. In the other three they were open-pollinated. The major portions of the data have been obtained on  $F_3$  and  $F_4$  progenies grown at Altona in 1948 and 1949.

Data on pollen abortion and seed set were secured on all plants available. Cytological observations were made on selected plants only, usually those of highest and lowest pollen abortion in each progeny. The data on pollen abortion and seed set will be considered first. They will be discussed in two groups, first those of families 1 and 2 which came from the two self-

pollinated  $F_1$  plants followed by the data for families 3 and 4 from two  $F_1$  open-pollinated plants. Detailed data for family 5 will not be presented since they repeat to some extent those of other families.

The frequency distributions and means for pollen abortion and seed set for progenies of families 1 and 2 from the two different self-pollinated  $F_1$  plants and from the Dakold  $S_3$  are given in Table 13. The combined data for all progenies of the C.P. 14 parent are included for comparison purposes. All progenies in these two families came from self-pollinated parent plants.

Considering first the pollen abortion data, it is seen that all progenies had a higher mean abortion than the Dakold  $S_3$ . The difference was significant at the one per cent level in most instances. There was also a number of progenies with higher mean sterility than the C.P. 14 inbred, the difference being significant for  $F_1$  progeny 40 and  $F_3$  progenies 63 and 66, the latter two coming from  $F_2$  plants having 95 per cent pollen abortion.

The two  $F_2$  progenies 40 and 41 are significantly different in mean pollen abortion, suggesting that either or both of the parents may have been heterozygous. The data on the C.P. 14 parent presented earlier indicate that it may have been segregating. It is probable that C.P. 9 was segregating also. In spite of this fact, the behaviour in  $F_2$  and  $F_3$  within a family tracing back to a single  $F_1$  plant can be used for a study of inheritance without this acting as a complicating factor.

In family 1 three out of the six  $F_2$  plants of progeny 40 were classified as male sterile and one other as 95.0 per cent or close to male sterile. In the  $F_3$  self progeny 63, which came from the 95.0 per cent sterile  $F_2$  plant, six out of the 13 plants were male sterile. The appearance of one plant with only 15.0 per cent sterility in the  $F_3$  progeny as well as the scarcity of low sterile plants in the  $F_2$  progeny suggest that low sterility was recessive in this family.

The behaviour in family 2, which descended from the other self-pollinated  $F_1$  plant was much different. There was a preponderance of low sterile plants in this family as a whole, most of them being in sterility classes of 35.0 per cent or lower. Eight out of 12 plants in the  $F_2$  progeny 41 were in the 25.0 per cent class or lower and only one was classified as male sterile. In this family, two out of three  $F_3$  progenies, 2-65 and 2-67, from  $F_2$  plants of low sterility had most of the plants in the lower sterility classes, while the third, 2-64, was apparently segregating for high sterility, having two plants classified as male sterile and two others in the 75.0 per cent class. The fourth  $F_3$  progeny, 2-66, which came from an  $F_2$  parent with high sterility, had all its plants in the higher sterility classes four out of the seven being classified as male sterile. The data from family 2 are not extensive, but they suggest that low pollen sterility was dominant in inheritance, in contradiction to those from family 1, which suggested the dominance of high sterility.

As to mean per cent seed set, all the  $F_3$  progenies of families 1 and 2 were lower than the Dakold  $S_3$ , ranging from 51 to 66 per cent as compared with 76 per cent. Certain of the differences were significant at the one per cent level, for example that for progeny 65, while others were not significant, such as that for progeny 64. The differences between the progenies them-

TABLE 13.—FREQUENCY DISTRIBUTIONS OF INDIVIDUAL PLANTS FOR PER CENT POLLEN ABORTION AND PER CENT SEED SET IN FAMILIES 1 AND 2 DESCENDING FROM F<sub>1</sub> SELF-POLLINATED PLANTS OF CROSS C.P. 9 × C.P. 14 F<sub>2</sub> AND F<sub>3</sub> PROGENIES GROWN IN 1947 AND 1948, RESPECTIVELY. ALL PROGENIES PRODUCED BY SELF-POLLINATION

Gen.	Family	Progeny	Parent abortion	Pollen abortion classes											Means			
				2.5	7.5	15.0	25.0	35.0	45.0	55.0	65.0	75.0	85.0	95.0	M.S.	Observed	Transformed	
F <sub>2</sub>	1	40	—	—	—	—	1	—	—	—	1	—	—	—	1	3	79.2	70.8 ± 10.54
F <sub>2</sub>	1	63	95.0	—	—	1	—	3	—	—	2	—	—	—	—	6	69.6	63.7 ± 7.42
F <sub>2</sub>	2	41	—	—	4	—	3	1	—	—	1	—	—	—	1	1	35.2	36.3 ± 5.98
F <sub>2</sub>	2	64	25.0	—	2	3	—	2	2	—	2	1	—	—	—	2	45.4	43.6 ± 6.74
F <sub>2</sub>	2	65	25.0	—	1	4	3	3	1	—	—	—	—	1	—	—	34.2	31.9 ± 3.59
F <sub>2</sub>	2	66	95.0	—	—	—	—	—	—	—	1	—	—	1	4	—	85.0	69.1 ± 4.34
F <sub>2</sub>	2	67	15.0	—	6	4	1	1	—	—	—	1	—	—	—	—	17.7	23.6 ± 3.07
Line C.P. 14	—	—	—	—	4	16	7	8	4	—	5	4	—	—	1	—	33.0	34.3 ± 1.93
Dakold S <sub>4</sub>	—	—	—	12	8	3	—	—	—	—	—	—	—	—	—	—	5.9	13.3 ± 1.03
Seed set classes																		
F <sub>2</sub>	1	63	—	—	—	—	—	3	1	1	—	2	1	—	—	—	54.0	47.5 ± 4.19
F <sub>2</sub>	2	64	—	—	—	—	1	1	—	2	2	1	3	—	—	—	62.0	52.2 ± 3.66
F <sub>2</sub>	2	65	—	—	—	—	—	—	—	—	2	3	—	—	—	—	51.2	45.5 ± 3.04
F <sub>2</sub>	2	66	—	—	—	—	—	—	—	—	1	1	1	—	—	—	66.4	54.7 ± 2.82
F <sub>2</sub>	2	67	—	—	—	—	—	1	1	—	4	—	—	—	1	—	57.2	49.8 ± 4.93
Line C.P. 14	—	—	—	—	—	2	1	2	2	2	8	6	10	3	—	—	59.6	50.7 ± 1.88
Dakold S <sub>4</sub>	—	—	—	—	—	—	—	—	—	2	2	3	6	6	4	—	76.0	62.0 ± 2.55

TABLE 14.—FREQUENCY DISTRIBUTIONS OF INDIVIDUAL PLANTS FOR PER CENT POLLEN ABORTION AND PER CENT SEED SET IN FAMILIES 3 AND 4 DESCENDING FROM F<sub>1</sub> OPEN-POLLINATED PLANTS OF CROSS C.P. 9 × C.P. 14. F<sub>2</sub>, F<sub>3</sub> AND F<sub>4</sub> PROGENIES GROWN IN 1947, 1948 AND 1949, RESPECTIVELY

Gen.	Family	Progeny	Parent	Pollen abortion classes											Means		
															Observed	Transformed	
				2.5	7.5	15.0	25.0	35.0	45.0	55.0	65.0	75.0	85.0	95.0			M.S.
F <sub>2</sub> *	3	42	—	1	1	—	—	—	—	—	—	—	—	—	—	29.2	30.8 ± 11.62
F <sub>3</sub>	3	68	65.0	—	—	—	—	—	—	—	—	—	—	—	—	53.3	48.7 ± 6.07
F <sub>3</sub>	3	69	15.0	1	2	3	—	—	—	—	—	—	—	1	—	35.3	36.1 ± 7.47
F <sub>3</sub>	3	70	7.5	3	1	—	—	1	—	—	—	—	—	1	—	34.6	34.7 ± 8.99
F <sub>4</sub>	3	50	15.0	—	—	—	1	—	—	—	—	—	—	—	1	56.7	54.0 ± 18.32
F <sub>4</sub>	3	51	—	2	5	—	2	—	—	1	—	—	—	—	—	17.5	22.5 ± 3.95
F <sub>4</sub>	3	53	7.5	1	—	—	—	—	—	—	—	—	—	—	—	35.0	35.7 ± 7.25
F <sub>4</sub> *	4	116	65.0	—	2	—	1	2	—	—	—	—	—	2	5	69.6	63.5 ± 8.02
F <sub>4</sub>	4	79	25.0	—	—	3	2	—	—	—	—	—	—	2	3	59.1	55.1 ± 9.05
F <sub>4</sub> *	4	80	M.S.	3	—	—	—	—	—	—	—	—	—	3	1	52.9	48.0 ± 7.77
F <sub>4</sub> *	4	54	M.S.	1	1	—	—	—	—	—	—	—	—	—	2	82.1	73.9 ± 12.17
F <sub>4</sub>	4	55	95.0	—	1	1	—	—	—	—	—	1	—	1	5	78.5	70.1 ± 8.27
F <sub>4</sub>	4	56	2.5	—	—	2	2	—	—	—	—	—	—	1	1	52.2	48.4 ± 9.03
F <sub>4</sub>	4	57	15.0	—	—	1	1	1	1	1	1	1	—	1	5	74.6	66.4 ± 6.89
F <sub>4</sub>	4	58	25.0	—	—	1	1	—	—	1	—	—	—	—	4	70.6	64.0 ± 9.24
F <sub>4</sub>	4	59	7.5	—	—	—	2	—	—	—	—	—	2	1	2	70.7	62.4 ± 9.57
F <sub>4</sub>	4	60	7.5	—	—	—	—	—	—	—	—	2	1	1	4	83.0	71.4 ± 5.76
F <sub>4</sub> *	4	61	95.0	—	2	1	2	1	—	—	—	—	—	1	4	65.3	59.1 ± 7.44
Line C.P. 14	—	—	—	—	4	16	7	8	4	5	4	2	—	1	—	33.0	34.3 ± 1.93
Dakold S <sub>3</sub>	—	—	—	12	8	3	—	—	—	—	—	—	—	—	—	5.9	13.3 ± 1.03

\* Produced by open-pollination, all others by selfing.



TABLE 14.—FREQUENCY DISTRIBUTIONS OF INDIVIDUAL PLANTS FOR PER CENT POLLEN ABORTION AND PER CENT SEED SET IN FAMILIES 3 AND 4 DESCENDING FROM F<sub>1</sub>, OPEN-POLLINATED PLANTS OF CROSS C.P. 9 × C.P. 14. F<sub>2</sub>, F<sub>3</sub> AND F<sub>4</sub> PROGENIES GROWN IN 1947, 1948 AND 1949, RESPECTIVELY.—*Concluded*

Gen.	Family	Progeny	Parent	Seed set classes										Means		
				2.5	7.5	15.0	25.0	35.0	45.0	55.0	65.0	75.0	85.0	95.0	Observed	Transformed
F <sub>3</sub>	3	68	—	—	—	2	—	1	—	1	—	1	1	—	46.5	42.8 ± 6.70
F <sub>3</sub>	3	69	—	2	—	1	—	—	—	—	—	—	—	—	12.0	16.6 ± 6.16
F <sub>3</sub>	3	70	—	1	—	—	2	1	—	—	—	—	—	—	28.8	31.1 ± 6.11
F <sub>4</sub>	3	50	38.5	—	—	—	—	—	—	—	—	—	—	—	3.1	9.2 ± 2.26
F <sub>4</sub>	3	51	—	—	1	—	—	2	5	2	—	—	1	1	62.0	52.7 ± 3.18
F <sub>4</sub>	3	53	—	—	—	—	—	—	—	—	—	—	—	—	19.3	26.7 ± 8.89
F <sub>3</sub>	4	79	—	—	—	—	—	—	—	3	2	—	1	1	66.7	55.4 ± 3.61
F <sub>3</sub>	4	80	—	—	—	—	2	—	—	1	1	3	2	—	51.6	45.9 ± 3.65
F <sub>4</sub> *	4	54	25.2	—	—	—	—	—	—	1	1	—	2	1	80.3	65.4 ± 1.59
F <sub>4</sub> *	4	55	65.1	1	—	—	—	1	2	2	2	1	1	—	51.3	45.2 ± 4.73
F <sub>4</sub>	4	56	—	3	—	1	2	—	—	1	1	—	—	—	17.9	21.6 ± 6.02
F <sub>4</sub>	4	57	72.8	—	—	1	1	—	—	4	2	1	1	—	36.9	40.8 ± 6.89
F <sub>4</sub>	4	58	63.7	1	—	1	2	2	2	3	—	—	—	—	29.9	32.3 ± 3.38
F <sub>4</sub>	4	59	28.6	1	1	1	—	1	2	1	2	1	—	—	32.2	32.4 ± 6.57
F <sub>4</sub>	4	60	45.9	1	1	1	4	3	1	1	—	—	—	—	25.2	31.0 ± 2.56
F <sub>4</sub>	4	61	51.0	—	1	1	—	1	—	1	2	6	4	—	62.8	52.6 ± 1.95
Line C.P. 14	—	—	—	—	2	—	1	2	2	8	6	6	10	3	59.6	50.7 ± 1.88
Dakold S <sub>1</sub>	—	—	—	—	—	—	—	—	—	2	2	3	6	6	76.0	62.0 ± 2.55

\* Produced by open-pollination, all others by selfing.

selves and in comparison with the C.P. 14 parent were not significant. The frequency distribution of the plants was fairly similar for all progenies and indicated little if any segregation except possibly progenies 63 and 67. No data are available on seed set of the parent plants but it is of interest to note that progeny 63, which came from an  $F_2$  plant almost male sterile, had virtually the same mean seed set as progenies 65 and 67 which came from plants of low pollen abortion. These results are an indication that the factors responsible for pollen abortion did not have an equivalent effect in reducing seed set.

Next the pollen and seed set data of families 3 and 4 will be discussed. These families descended from open-pollinated  $F_1$  plants in contrast to families 1 and 2 described above which descended from self-pollinated  $F_1$  plants.

The data on pollen sterility of the  $F_2$  progenies from the two open-pollinated  $F_1$  plants are in Table 14: 3-42 and 4-116. Also included are the data of progeny from selected  $F_2$  and  $F_3$  plants, most of which were self-pollinated. The mean pollen sterility for all progenies was higher than for Dakold  $S_3$  the difference being significant at the one per cent level in most instances. Most progenies were also higher in pollen abortion than the C.P. 14 parent, especially those for families 4 where the differences were significant in several instances, indicating that some of the factors responsible for the high sterility came into the cross from either or both parents; in this case either C.P. 9 or the pollen source for the open-pollination.

Considering the frequency distributions (Table 14) of the progenies from the open-pollinated  $F_1$  plants which were sibs of the parent plants of families 1 and 2 discussed above family 3 progeny 42 appeared to have a low frequency of male sterile or highly sterile plants while family 4 and progeny 116 had high frequencies. This difference was consistent for the later generations of the two families also.

For family 3, the  $F_3$  progeny 68 from a 65 per cent sterile  $F_2$  plant had five out of nine plants in the relatively low pollen sterile class of 35.0 per cent and only one in the high male sterile class. The other five  $F_3$  and  $F_4$  progenies in this family, four of which are known to have come from parents of low pollen sterility (3-69, 3-70, 3-50 and 3-53) had the majority of their plants in the lower pollen sterility classes with only an occasional one in the male sterile or highly sterile classes. The  $F_4$  progeny 51 for which the parent sterility is not known had much the lowest mean, two out of 11 plants being in the 2.5 per cent class which could be considered normal. These results suggest that pollen abortion was a dominant character in family 3 unless the variation does not represent segregation.

In family 4 the  $F_2$  progeny 116 (Table 14) ranged from 7.5 per cent pollen sterility to the male sterile class with five out of 14 of the plants in the 35.0 per cent class or lower. In  $F_3$  and  $F_4$  generations two progenies, 80 and 54, from male sterile  $F_2$  and  $F_3$  parent plants, respectively, the former self-pollinated and the latter open-pollinated, and two other progenies, 55 and 61, from 95.0 per cent sterile  $F_3$  parents, both open-pollinated, had a preponderance of highly sterile or male sterile plants, but also included plants of low sterility, suggesting that low sterility was recessive and was appearing as a result of segregation. It also appeared

that the plant classified as male sterile which was the parent of progeny 80 produced some functional pollen in that it yielded seed when selfed.

Except for progeny 56, the other  $F_3$  and  $F_4$  progenies of family 4 (Table 14), which all came from relatively low sterile plants that were self-pollinated, had at least one-half of their plants in the high sterility classes many being male sterile. Of these progenies which came from relatively low sterile  $F_3$  plants,  $F_4$  progeny 56 had a higher relative frequency of low sterile plants than did the others. The others had relatively high frequencies of plants with high pollen sterility. These did not appear to differ greatly from the  $F_3$  and  $F_4$  progenies from highly sterile  $F_2$  and  $F_3$  parent plants discussed in the preceding paragraph, although this may have been the result of open-pollination since three out of the four unfortunately did not yield self progenies.

In family 4 some plants were phenotypically dominant for low sterility and appeared to segregate for high sterility. Others were phenotypically dominant for high sterility and appeared to segregate for low sterility. One apparent exception was self progeny 60, which had no plants in the low sterility classes, although it came from a low sterile  $F_3$  plant. This parent plant was phenotypically low sterile but evidently genotypically high sterile. The behaviour may also indicate that pollen abortion is highly sensitive to environment.

As to seed set, the data from families 3 and 4 (Table 14) were similar to those for the families 1 and 2 from self-pollinated  $F_1$  plants, in that all progenies except 54 had a lower mean per cent seed set than the Dakold  $S_3$ . For most of the progenies the differences were significant and in many instances at the one per cent level whereas several of the differences for families 1 and 2 were not significant (Table 13). In contrast to families 1 and 2 there were a number of significant differences between progenies and also between the progenies and the C.P. 14 parent. These differences were largely due to the appearance of a number of plants with very low seed set in families 3 and 4. This extremely low seed set appears to be inherited since it occurred in one  $F_3$  and one  $F_4$  progeny, 3-69 and 3-50, respectively, both selfed. In these and in  $F_4$  progeny 4-56 also selfed, all or most of the plants had less than 35.0 per cent seed set. Also its occurrence in  $F_4$  progenies 55, 56, 58, and 60 in family 4, all selfed progenies, except 55, three of which are known to have come from parents with moderate to high seed set suggests that the condition was recessive. The lack of any plants in the two lowest classes in the C.P. 14 parent material also suggests that the condition came from the C.P. 9 parent or was a result of the open-pollination in  $F_1$ .

An  $F_2$  highly pollen sterile segregate of family 4 was crossed in 1947 to a plant with low pollen abortion in the Dakold  $S_3$  line. The  $F_1$  of this cross was grown at Altona in 1948. This and the resulting progenies will be referred to as family 6, the data for which are in Table 15.

Seven out of 11 plants in the  $F_1$  of family 6 were in the highly sterile classes and two others were in the lowest sterility classes, suggesting dominance of high pollen sterility although there was segregation also.

Of the two lowest pollen sterile  $F_1$  plants in family 6 one was crossed reciprocally with an unrelated plant of low pollen abortion, giving progenies





8 and 10 (Table 15). The unrelated plant was an  $F_1$  of a cross of two plants selected for low pollen abortion in a Dakold culture selfed two generations. The behaviour of progenies 8 and 10 (Table 15) from the reciprocal cross was greatly different. Progeny 8, in which the  $F_1$  of family 6 was the male parent, had all but one of its 16 plants in the 2.5 per cent pollen sterility class that one being in the 7.5 per cent class. Progeny 10, in which the same  $F_1$  of family 6 was the female parent had five of its 15 plants in the male sterile class or close to male sterile and five in the 2.5 per cent class with the remaining five scattered but all in the 35.0 per cent class or lower. This difference in behaviour of the reciprocal crosses is evidence for cytoplasmic inheritance of this sterility. The second  $F_1$  of low pollen abortion in family 6 was crossed as a female parent with another unrelated plant of low pollen abortion, but of the same origin, giving progeny 9. This was similar to progeny 10 in that it also had two distinct modal classes at the pollen abortion extremes 2.5 per cent and male sterile, only two plants of its 15 being in intermediate classes. This indicates segregation for one or more recessive genes for the control of sterility.

The two low pollen sterile  $F_1$  plants of family 6 used in the crosses discussed in the previous paragraph gave  $F_2$  self progenies 23 and 24 (Table 15). As in progenies 9 and 10 resulting when they were used as female parents in crosses there were two distinct modal classes at the sterility extremes. This indicates segregation for one or more recessive genes for the control of sterility in addition to the cytoplasmic effect in the material of this family from the C.P. 9  $\times$  C.P. 14 cross. In the other  $F_2$  progenies of family 6, one (No. 44) from an  $F_1$  male sterile plant consisted of three male sterile plants. Another  $F_2$  progeny (No. 46) from an  $F_1$  plant with relatively low pollen abortion had seven of its 15 plants in the male sterile class and the remainder scattered over a wider range than for the other  $F_2$  progenies but five were in the 25.0 per cent class or lower. These results also indicate segregation. The existence of two distinct modal classes at the extremes of the sterility range suggests that the number of genes controlling the sterility was small.

The data on per cent seed set for family 6 in Table 15 are different from those of the other families. Five of the nine progenies (8, 9, 10, 23 and 24) had a higher mean seed set than the Dakold  $S_3$ , but the difference was significant only for progeny 9. Of the four which were lower the difference was also significant in only one instance, progeny 46. The  $F_1$  progenies 8, 9 and 10, and the two  $F_2$  self progenies 23 and 24 from  $F_1$  plants with high seed set were relatively uniform in having high seed sets. Some segregation was indicated by the bimodal distribution in the one  $F_2$  progeny 46 which came from an  $F_1$  with lower seed set. These data suggest that the factors for high pollen abortion and low seed set have been separated to a large degree and that mainly the former are present in family 6 in the  $F_1$  and  $F_2$  progenies.

The correlation coefficients for the association of per cent pollen sterility and per cent seed set were calculated for 27 of the progenies of the C.P. 9  $\times$  C.P. 14 cross. Five of these values were positive and the remainder negative. Generally they were of low order. Only two exceeded the one per cent level and two others the five per cent level

TABLE 16.—MEIOTIC BEHAVIOUR AND PER CENT POLLEN ABORTION, SEED SET AND ABNORMAL QUARTETS OF PLANTS FROM CROSS OF SELFED LINES C.P. 9  $\times$  C.P. 14

Year, progeny and plant	Per cent pollen abortion	Per cent seed set	Per cent abnormal quartets	Meiotic behaviour
				<i>Family No. 1</i>
47-40-5	95.0	—	2.5	Two univalents in 5 per cent of cells. Metaphase to quartets in same anther.
48-63-3	75.0	81.6	1.9	Two univalents in a few cells. Several stages in one another.
				<i>Family No. 2</i>
47-41-9	25.0	—	4.5	One or two univalents in a few cells. Diakinesis to telophase I in same anther.
48-64-5	M.S.	74.6	7.6	Chromosomes shorter than normal at metaphase and rare rod bivalent.
48-65-2	25.0	55.2	10.9	Six normal pairs and two short pairs at diakinesis, excess of rod bivalents and stretched rod bivalents at metaphase, few cells with long bridge and fragment at anaphase.
48-65-5	15.0	40.0	2.7	Diakinesis similar to plant 65-2 except one cell observed with a single chromosome not paired.
48-65-6	85.0	24.4	4.0	About 25 per cent of cells suggest poor orientation at metaphase plate otherwise normal.
				<i>Family No. 3</i>
47-42-8	65.0	—	8.0	Two univalents in 5 to 10 per cent of cells, lagging at anaphase II.
47-42-14	7.5	—	12.0	Similar to plant 42-8 but two cells noted with bridge and fragment at anaphase I.
48-68-4	M.S.	24.3	11.4	Two to four univalents frequent, four or five cells in which chromosomes were in three groups at metaphase instead of normal central plate.
48-68-6	35.0	60.0	58.7	About 50 per cent of cells at division I forming an interphase of three cells instead of two. Metaphase I chromosomes in such cells in three groups suggesting a tripolar spindle: evidence that cell wall forming the three cells was laid down before or after anaphase I. Quartets varied from normal to six cells.

TABLE 16.—MEIOTIC BEHAVIOUR AND PER CENT POLLEN ABORTION, SEED SET AND ABNORMAL QUARTETS OF PLANTS FROM CROSS OF SELFED LINES C.P. 9  $\times$  C.P. 14—*Concluded*

Year progeny and plant	Per cent pollen abortion	Per cent seed set	Per cent abnormal quartets	Meiotic behaviour
<i>Family No. 3—Concluded</i>				
48-69-1	15.0	0.1	50.0	Diakinesis to division II in same anther. Two univalents in 25 per cent of cells, tripolar condition but not as frequent as in plant 68-6.
48-70-1	45.0	30.4	17.2	One or two univalents in 20 per cent of cells. Three stages in one anther.
48-70-2	95.0	5.2	8.5	Few metaphase figures seen indicate univalents frequent. Three stages in one anther.
<i>Family No. 4</i>				
47-116-10	95.0	—	0.0	Normal except for one cell showing a bridge and fragment at anaphase I.
48-80-1	M.S.	25.2	75.6	Very high frequency of univalents, up to 10 in some cells, bivalents usually rod type only.
48-80-2	95.0	65.1	1.6	Normal except for few cells with long bridges at anaphase I.
48-80-6	7.5	28.6	7.5	Normal (seed set basis only 44 florets.)
49-59-13	95.0	12.5	83.0	Long chromosomes at metaphase, similar to selfed line C.P. 11 (seed set basis only 48 florets.)
<i>Family No. 6</i>				
48-18-3	95.0	38.0	1.9	Normal except for low frequency of rod bivalents.
48-18-4	2.5	94.1	1.6	Normal.
48-18-6	M.S.	68.0	1.0	Normal except for low frequency of rod bivalents.

required for significance. As a group, such a large proportion of negative values may be taken as indicative of a slight trend for the same factors to have caused reduced seed set and pollen abortion but certainly no significant or strong association was demonstrated. Apparently the factors which caused pollen abortion were not equally effective in reducing seed set.

The association of per cent normal quartets with per cent pollen abortion and seed set was studied by calculation of correlation coefficients in families 2, 3, 4 and 6. Insufficient plants were examined cytologically in the other families to warrant any calculations. These correlations were of a similar order to those for the association of pollen abortion and seed set. Only one approached the five per cent level of significance, that for per cent normal quartets and seed set in family 4. The values for the association with pollen abortion were all negative and those for seed set all positive, which may indicate the existence of a slight association between cytological irregularity and both microspore abortion and reduced seed set in this material. This statement is supported by cytological behaviour at meiosis, in which some plants were found with sufficient irregularity to account for the sterility.

The meiotic behaviour and other relevant data for individual plants of interest from each family are shown in Table 16. In most instances the plants of one year were the parents of those grown the following year.

The data of Table 16 show that plants with relatively normal cytological behaviour may have high pollen sterility, e.g. 48-63-3 and 48-18-6. Both of these plants had good seed set. There were those with both high pollen abortion and low seed set, yet normal or nearly normal cytological behaviour. Examples of such plants were 48-65-6 (family 2) and 48-18-3 (family 6). The behaviour of these plants was an indication that genic complexes which did not affect meiosis caused either microspore abortion or reduced seed set or both.

There were a number of instances in which the cytological irregularity could account for the high sterility observed, such as plants 48-68-4 and 48-68-6 in family 3, or 48-80-1 and 49-59-13 of family 4. Even among these plants, however, there were some anomalous situations such as the pairs of plants 48-68-4 and 48-68-6 of family 3 and 48-70-1 and 48-70-2 also of family 3, in which both the abortion of the pollen and the seed set were the reverse of that expected from the percentage of abnormal quartets.

Considering the plants within individual families, there was evidence in some instances of inherited meiotic irregularity in that the same abnormality appeared in the parent plant and its progeny or in two sib progenies. Examples of this behaviour were the presence of several stages in one anther, in plant 47-40-5, and its daughter plant 48-63-3 of family 1 and the appearance of the three cell interphase or tripolar spindle condition in the  $F_3$  progenies 68 and 69 of family 3 (Figure 6). Similar behaviour was also evident for the condition of six bivalents and two paired fragments at diakinesis (Figure 4), reported for progeny 65 in family 2 and progeny 67 of the same family, the latter not being mentioned in Table 16.

The other most significant feature was the appearance, in a number of progenies, of meiotic irregularities which were not present in the parent plants. Among these was the case of six bivalents and two paired frag-



ments which occurred in progeny 65 and not in the parent plant 47-41-9 of family 2, though such behaviour was noted in line C.P. 14. Another was the tripolar spindle condition of progenies 68 and 69 of family 3 which was not present in parent plants 47-42-8 and 47-42-14, respectively. A third was the long chromosome condition of plant 49-59-13 family 4. Its parent plant 48-80-6 was normal. A further instance not recorded in Table 16 was the discovery of a translocation in a plant of family 5, when the selfed parent was normal.

The six bivalents plus two paired fragments and the tripolar condition could have been due to recessive genes being brought into homozygous condition by selfing. The fact that these conditions existed in two small progenies, in each of their respective families, suggests that if the condition were due to genic action the number of gene differences was small and most likely only one. The same can be said for "long chromosomes" in plant 59-13 of family 4.

The occurrence of the translocation in family 5 can most probably be explained by a break and rearrangement of two pairs of chromosomes occurring in the parent plant. The possibility of pollen contamination cannot be ruled out, as plants known to possess translocations were growing five to ten feet from the parent plant. The translocation has also been identified as free of the nucleolus which conforms with those which could have been a contamination source.

In summary the data from the C.P. 9  $\times$  C.P. 14 cross show that the causes for pollen sterility and reduced seed set in this cross were varied and complex. Comparisons with the C.P. 14 inbred suggest that most of the factors responsible for the high sterility came from the other parent, the C.P. 9 inbred line. It is unfortunate that more information is not available on that line. The genetic data suggest that both dominant and recessive factors may have been responsible for the high pollen sterility. There is also the added complication of cytoplasmic control from the data on one reciprocal cross in family 6. The lack of highly significant correlations between per cent pollen abortion and per cent seed set in the different progenies shows that the factors responsible for pollen abortion and reduced seed set were largely independent.

Cytological abnormality as observed in the microsporocytes could explain the high spore abortion and low seed set of some plants. Seed set in most cases was far from complete fertility and might be due to any of the abnormalities. It cannot be assumed, however, since there were plants with normal cytology but high pollen abortion that the observable cytological aberrations were entirely responsible for microspore abortion and low seed set in these plants. Furthermore, the non-significant correlations of pollen abortion and seed set with quartet behaviour as a measure of cytological behaviour, did not suggest any strong association. There was some evidence that the cytological abnormalities were inherited and probably on a simple basis. If they were inherited they should have appeared in the parental inbreds. Most of the gross abnormalities were not found in the inbreds and were not discovered until  $F_3$  in the cross. It seems possible therefore that in the C.P. 9  $\times$  C.P. 14 cross a condition may have been set up causing chromatin and genetic instability resulting in

TABLE 17.—PER CENT POLLEN ABORTION AND SEED SET IN CROSSES AND OTHER MATERIAL TESTED FOR SOURCES OF LINES UNIFORM FOR LOW POLLEN ABORTION AND HIGH SEED SET

Number	Year	Gen.	Pollen abortion			Plants of progenies		
			♀ Parent		♂ Parent	n	Observed range	Mean
			Source	Per cent	Source			
11	1948	F <sub>1</sub>	1947-93-16	2.5	1947-90.4	11	2.5-7.5	3.1
19	1948	F <sub>1</sub>	1947-97-3	2.5	1947-93.2	15	2.5-2.5	2.5
20	1948	F <sub>1</sub>	1947-93-2	2.5	1947-97.3	15	2.5-2.5	2.5
1	1949	F <sub>1</sub>	1948-85-20	2.5	1948-20.8	16	2.5-7.5	3.8
2	1949	F <sub>1</sub>	1948-20-8	2.5	1948-85-20	15	2.5-7.5	4.2
7	1949	F <sub>1</sub>	1948-11-3	2.5	1948-14.6	16	2.5-7.5	4.1
8	1949	F <sub>1</sub>	1948-14-1	2.5	1948-18.4	16	2.5-7.5	2.8
19	1949	S <sub>4</sub>	1948-85-20	2.5	Selfed	11	2.5-15.0	10.2
22	1949	F <sub>2</sub>	1948-11-3	2.5	Selfed	12	2.5-7.5	3.8
85	1948	S <sub>3</sub>	Dakold*	—	—	23	2.5-15.0	5.9
Seed set								
11	1948	F <sub>1</sub>	1947-93-16	—	1947-90.4	4	70.3-91.4	85.9
19	1948	F <sub>1</sub>	1947-97-3	—	1947-93.2	7	68.3-119.0	95.7
20	1948	F <sub>1</sub>	1947-93-2	—	1947-97.3	7	83.5-97.3	90.8
1	1949	F <sub>1</sub>	1948-85-20	89.9	1948-20.8	16	86.7-107.8	96.2
2	1949	F <sub>1</sub>	1948-20-8	97.3	1948-85-20	16	91.0-102.9	98.7
7	1949	F <sub>1</sub>	1948-11-3	70.3	1948-14.6	16	54.2-92.8	81.2
8	1949	F <sub>1</sub>	1948-14-1	—	1948-18.4	16	70.6-95.0	85.0
19	1949	S <sub>4</sub>	1948-85-20	89.9	Selfed	11	81.3-99.3	87.6
22	1948	F <sub>2</sub>	1948-11-3	70.3	Selfed	11	52.7-84.2	71.0
85	1948	S <sub>3</sub>	Dakold*	—	—	23	42.8-101.0	76.0

\* The same progeny as used for comparison in other parts of this study

frequent mutation, as well as observable and possibly unobservable cytological abnormalities. Such a condition could also be the expression of the genic complement which has been established by crossing lines C.P. 9 and C.P. 14.

### *Development of a Low Sterility Line*

The objective in this portion of the work has been to develop a line uniform and pure breeding for pollen abortion, not higher than the 7.5 per cent class and preferably with uniformly high seed set also. The major effort has been through crossing plants with low pollen abortion. Where possible, self progenies of these plants were also grown but usually the plants chosen failed to set seed when isolated with a parchment bag. The data on pollen sterility and seed set of progenies of the most promising crosses, together with progenies of some of the parent plants and other plants which were selected for low pollen abortion are presented in Table 17.

Of the material listed in Table 17, the most promising was progeny 11—1948. It consisted of a cross between a Dakold plant from two years of selfing and one of inbred line C.P. 2. The mean pollen abortion was lower and seed set higher than in the Dakold  $S_3$  progeny. Also it has produced a self progeny 22—1949, a rare occurrence, in which the pollen abortion was low. Further, when the parent of this self progeny was used in another cross it yielded progeny 7—1949 which also had low pollen abortion and higher seed set than the Dakold  $S_3$ . The undesirable feature was that the self progeny produced 34 normal and 10 albino seedlings.

The next most interesting lots were progenies 19 and 20—1948 which comprised a reciprocal cross between plants 1947, 93-2 and 97-3 in Dakold progenies selfed two and three generations, respectively. These two progenies, 19 and 20—1948, had uniformly low pollen abortion and relatively high seed set. Their unfortunate feature was that they set no seed when selfed, even though two or more spikes were enclosed in each selfing bag, so that they could not be inbred by this method. Remnant seed is available. It may be possible to inbred these progenies in future by sib-pollination in hopes of propagating, in a uniform line, the low sterility which they exhibit.

Progenies 1 and 2 were amongst the lowest in pollen abortion discovered and had the highest seed set reported in Table 17. The one parent tested, 85-20 (progeny 19), has given plants with 15.0 per cent abortion which, even though higher than desired, is still of a low order. However, genetic variability was indicated in this progeny through the appearance of 37 normal and 13 albino seedlings. The other parent came from progeny 20—1948 already mentioned as being highly desirable. There is a good quantity of self seed available for continuing progenies 1 and 2.

Progeny 8—1949 will be recognized as one of those of family 6 of the C.P. 9  $\times$  C.P. 14 cross. The reciprocal cross produced high pollen abortion. If the hypothesis that the pollen abortion in this family was conditioned by the cytoplasm is correct, progeny 8 may yield lines uniform for low pollen abortion. Self seed is usually obtained in good quantities in the C.P. 9  $\times$  C.P. 14 material. Its parentage in progeny 8 may have brought in some



genes for self fertility. If so one barrier to producing a selfed line with low pollen abortion may be removed. Two out of three plants selfed in this progeny in 1949 produced good quantities of self seed.

### DISCUSSION

The inbred lines of rye used in these studies had been selected at University Farm, St. Paul, in the breeding project for three to thirteen generations when they were diallel crossed in 1940. All progenies examined came from at least five generations of selfing. Desirable agronomic characters such as vigour, winter hardiness, and uniform seed colour had been selected. The lines also had been selected for high seed setting when isolated under bags.

In previous studies Müntzing (20) has shown that reduced seed set, increased pollen abortion and larger cell size were associated with the presence of supernumerary chromosomes as described by himself and others. No supernumeraries were observed in the inbreds in this study but they were found in the progeny of three out of nine open-pollinated spikes selected for low seed set from an increase field of Emerald rye, a variety produced in 1927 by combining selected selfed lines.

There are a number of instances in which the pollen and ovule abortion or seed set are associated with cytological aberration, probably the best known among them is the translocation causing 50 per cent pollen and ovule abortion. It was first reported by Belling (6) in the velvet bean *Stizolobium deeringeanum* Bort. and later discovered in maize in a large number of instances (1). Beadle (4) reported a case of asynapsis in maize causing complete male sterility and 90 per cent female sterility. Pericentric inversions cause pollen and ovule abortion (13).

As translocations were not observed with any frequency except in the Emerald selections they have not been a factor in this study. The pericentric inversion can only be detected cytologically at pachytene, a stage which was not examined in this work, thus it may have been present and responsible for the high pollen abortion and low seed set in certain plants. This type of inversion would be a possible agent in the "cryptic structural hybridity" proposed by Stebbins *et al.* (31).

In only a few cases was there a relation between pollen abortion and seed set and in most cases no relation between either and cytological aberrations. Since among these three characters, only seed set had been selected during the inbreeding this lack of association may be understandable. As long as cytological aberrations, or factors affecting pollen abortion were such that they did not affect seed set there would be no selection against them.

A number of factors causing pollen abortion with no effect on ovule abortion have been observed. Small deficiencies, small translocations and paracentric inversions have little or no effect on the female side (9, 11, 29). Burnham has reported the *pa* gene in corn causing 50 per cent pollen abortion but no ovule abortion (10). Beadle studied 16 genetically different male steriles with normal female fertility in maize (5). Cytoplasmic male sterility has also been reported in several species (3, 12, 16, 26, 30). The evidence of inversions in lines C.P.<sub>2</sub> and C.P.<sub>14</sub> and of one translocation



involving short segments in line C.P.<sub>10</sub> may explain the lack of association between pollen and ovule abortion in certain of the material in the observations reported here. There was also the evidence of cytoplasmic control of pollen sterility independent of ovule abortion in one family of the C.P.<sub>9</sub> × C.P.<sub>14</sub> cross. As long as such factors had no marked effect on physiology of the plant or seed set they could easily have been carried in the lines during the inbreeding process prior to the commencement of this study.

Müntzing (19) has recorded significant differences between varieties of rye for pollen and female sterility. In other work (21) he found inter-plant variations and concluded genotypic differences must account for part of the sterility.

In the studies reported here there was evidence of genetic variability for pollen abortion within the inbred lines as shown by significant mean differences between progenies within the same line; and similar evidence, but less pronounced, for genetic variability in seed set. Often the differences were between progenies which had the same grandparental plant indicating that it had been heterozygous for factors affecting sterility even though selfed for several generations. While no conscious selection had been made for pollen condition it might normally be expected that the factors controlling it would be more homozygous after the continued selfing than the evidence indicates.

The interesting feature of the data from the inbreds is the high variability, especially in pollen abortion, within the inbred lines after so many generations of selfing. There is some evidence that inbreeding in other plants, as well as in rye, is accompanied by an increase in abnormal meiotic behaviour. In *Dactylis glomerata*, Myers (24) and Myers and Hill (25) found that the numbers of univalents and micronuclei increased as inbreeding progressed. Blanco (7) has reported greater meiotic aberration in inbreds of corn than in hybrid material. Clark (13) has also found evidence of chromosome rearrangement in inbreds of corn but studied a large population of plants to locate the few abnormalities reported. In rye, Lamm (17) found reduced chiasmata frequency and numerous meiotic disturbances in lines which had been inbred for seven to nine generations. Müntzing and Akdik (23) studying earlier generations of inbred material found meiotic irregularity much higher in the third generation than in the first generation. Such observations suggest the possibility that the reduction in vigour caused by inbreeding makes the chromosomes and the gametophyte generation highly sensitive to environment and may account for the variability in cytological behaviour and spore abortion found in the inbreds. The behaviour could also be due to recessive genes affecting chromosome behaviour being brought into homozygous condition by inbreeding.

Evidence of heterozygosity in inbred lines, possibly due to new mutations has been reported in corn by Jones (15) and in rye by Leith and Shands (18) but considering the great amount of inbreeding, particularly in corn, it would be expected that more frequent mention of this behaviour would appear in the literature if it were a common occurrence. It is understandable that a proportion of inbreds would be heterozygous after extended inbreeding due to chance alone or to new mutations but the



consistent variability and evidence of heterozygosity for the inbreds reported here is likely due to some other cause, the situation in rye being different from corn, the most extensively studied cross pollinated species.

In cross pollinated species, recessive abnormalities of many types are likely to accumulate. The number of such abnormalities would be in inverse relation to the self-fertility of the species. Corn is quite self-fertile while rye is quite self-sterile, though self-fertile lines have been secured by Brewbaker (8), Peterson (27) and Warren and Hayes (32) as well as those lines in this study. Because of the greater self-sterility in rye it might be expected that a larger number of abnormalities would occur in it than in corn. The reports of Müntzing and co-workers (2, 19, 28) indicated that this may be true as they found a relatively high frequency of pollen abortion and cytological abnormalities in unselected open-pollinated rye varieties.

In a species such as rye with only seven chromosomes homozygosity for a high proportion of these factors might occur quickly even if only a single chromosome were brought into the homozygous condition. Once homozygous, selection for characters controlled by the genes on it would have no further effect. Thus if there is a large number of recessive genes in rye capable of causing sterility and abnormal chromosome behaviour and a portion of them are brought into the homozygous condition in the early stages of inbreeding it may be expected that extensively inbred material will show the variability characterized by the selfed lines of this study. It might also be expected that because of abnormal chromosome behaviour inbred lines would remain heterozygous.

For a study of sterility in rye by the usual methods normal lines or lines uniform for low pollen abortion and high seed set are necessary. The development of such a line has been one of the objectives of this study. There are indications from the results so far obtained that this objective can be reached. Such a line may contribute greatly to an understanding of the causes of pollen abortion and reduced seed set in this species and ultimately to increase its economic value. Lines as normal in seed set and pollen fertility as barley or corn are the ultimate goal.

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## NOTE ON CURRENT STUDIES ON THE CHEMOTHERAPY OF AMERICAN FOULBROOD OF THE HONEYBEE AND ON THE STABILITY OF SULPHA DRUGS IN HONEY<sup>1</sup>

During the past summer a number of antibacterial agents (sulpha drugs and antibiotics) were tested against American foulbrood (AFB) of the honeybee in the apiary as part of a long-term project on the chemotherapy of bee diseases.

The pure compounds, or concentrates of these, were fed in sugar syrup, heavily inoculated with spores of AFB, to colonies of bees in the apiary as described in previous publications (1, 2). The colonies were examined periodically throughout the summer, complete freedom from infection of a colony being the criterion used for evaluating the efficacy of the compounds tested. Briefly, terracon, an impure concentrate of the antibiotic terramycin, when fed at the rate of 0.5 gram per gallon, per colony, completely controlled AFB. Magnamycin, a newly isolated antibiotic, was not effective. Sulmet, or sulphamethazine, a sulpha drug prepared for veterinary use, was completely effective against AFB; one tablet (0.5 gram) per gallon, per colony was sufficient for this purpose.

Stability in honey of compounds found to be effective against AFB is of considerable practical importance to the beekeeper. Three years ago an experiment was begun with three sulpha drugs to determine whether their anti-AFB activity would persist after storage in honey for several years. Accordingly each drug was mixed into honey at the rate of 2.0 grams per 20 pounds, 5 of which were then diluted with an equal weight of water, inoculated with AFB spores and fed at once, along with unmedicated inoculated control samples, and the remaining 15 pounds lots stored. Each subsequent summer 5 pounds of this medicated honey were tested in a similar manner. The results indicate that the three drugs tested, sodium sulphathiazole, sulphathiazole and sulphadiazine completely controlled AFB even after three years' storage in honey. Similar experiments are now under way with the antibiotic terramycin.

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— H. KATZNELSON and C. A. JAMIESON,  
Bacteriology Division, Science Service, and  
Apiculture Division, Experimental Farms  
Service, Department of Agriculture, Ottawa.

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